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OPTIMUM SOLUTIONS AS PHYSIOLOGICAL REFERENCE STANDARDS IN ESTIMATING NITROGEN UTILIZATION BY ASPERGILLUS NIGER[1]

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INTRODUCTION

The absence of biological reference standards, it is believed, has contributed to the difficulties in the physiological interpretation of the quantitative data of mineral nutrition. Though accurate control of the physical environment in laboratory studies with organisms has become frequent, the use of nutrient solutions physiologically indeterminate is still a routine procedure. Comparisons between the quantitative responses of organisms under these conditions would seem of lessened value since only one of the innumerable solutions that can be employed provides an accurate scientific basis for comparisons. This is the optimum nutrient solution for growth containing a minimum quantity of constituents (*12*),[2] and is the only one whose composition is subject to exact definition on the basis of physiological response. It is the nutrient solution whose every component serves most efficiently in the production of mass by the organism within a precisely defined physical environment.

The advantages to be derived from the use of optimum solutions will be readily apparent to the physiologist. The effects of nutrients under these conditions are no longer determined on the basis of absolute values, but become comparative and relative to the standard physiological effects produced at their optimum concentrations. Since the effects of a variation will always be relative to those obtained under standard conditions (optimum), they should be directly comparable. The same advantage should hold for comparisons between different organisms. Whether the results obtained at one level of carbohydrate nutrition are strictly comparable to those holding at another level with the same organism is an important consideration, however. Complete agreement between results at different levels of carbon nutrition would enhance the value of all data inasmuch as it would indicate their general significance in the interpretation of the organism-solution system.

This procedure has been employed in this brief study of the nitrogen nutrition of *Aspergillus niger* Van Tiegh. It was considered that the extent of agreement in results relative to the respective maxima of the optimum solutions would furnish an adequate test. The accumulation of experimental results, therefore, began with the study of the

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[2] Italic numbers in parentheses refer to Literature Cited, p. 731.

optima for the known mineral constituents in an ammonium nitrate solution at a practically optimum temperature, at three levels of carbohydrate nutrition. The effects of nitrogen concentration on yield, acidity of the culture solution, and sporulation were then obtained with each of these solutions. An analysis was made of the mineral constituents in the mycelial felts grown on the three optimum solutions; but only nitrogen was determined in the material obtained by variation of the nitrogen supply. Comparative experiments were performed with ammonium nitrogen and with nitrate nitrogen, except that analyses were omitted.

Brief reference, in addition, is made to studies on the theoretical equation for growth. The value of empirical growth equations for computing fertilizer requirements of plants has been recognized by Russell (*11*). Ojima (*8*) was able to compute the quantities of mineral nutrients absorbed. Special emphasis will be placed on the Mitscherlich growth formula (*11*) because of the attempts made to assign physiological meaning to its constants. Much of the discussion relating to this formula might have been avoided if it were possible to grow the higher plants so as to obtain yield data of high precision. Even under controlled environmental conditions (*14*), however, it is impracticable to obtain the necessary precision, because of the limited number of plants that can be grown in an artificial environment. The increased precision of control practicable with fungi has made possible an improvement in accuracy of physiological results.

Considerable space, therefore, has been devoted to the tabulation of data and the discussion of experimental precision as an important phase of general interest. Control of the physical environment and elimination of individual differences by the use of average values are responsible, no doubt, for much of the accuracy attained. An equally important contribution to precision has been made through control of the chemical environment. Purity of reagents is indispensable, of course, but it is equally important that each essential element be present in physiologically measured amount. Even though yields are maximum it is no exaggeration to assume that each essential element that participates in the development of the organism unknown to the investigator contributes directly to experimental inaccuracy, since the presence of each of these elements depends on impurities in the chemical compounds. Reliance on chance additions necessarily implies the use of fluctuating concentrations. It is doubtful, therefore, that the limit in precision has been reached, since it is almost certain that not all the chemical elements essential for the nutrition of *Aspergillus* are known.

Though the factors underlying the distribution of nutrients between organism and nutrient solution have received considerable attention, an adequate explanation of all phases of absorption has not as yet been accomplished (*7*). The ability of an organism to absorb a nutrient against a concentration gradient has been of special interest to investigators. Data are submitted, therefore, which indicate that relative solubilities of a compound in protoplast and nutrient solution may afford a partial explanation supplementing that given by the Donnan equilibrium. Computations of total nitrogen in the mycelial felts were made with considerable accuracy on the basis of an assumed constant protoplasmic nitrogen content and the distribution of the

remainder between organism and solution according to laws governing the distribution of a solute between two immiscible solvents.

No attempt will be made to review the literature bearing on the mathematical formulation of growth, in view of the detailed and thorough treatment of this subject by Russell (*11*) recently. Other citations will be mentioned in connection with the material to which they have reference. There is no experimental material available for a study of the effects on the growth of an organism of a graduated variation in a mineral constituent as compared with an optimum solution of minimum salt content.

METHODS

The cultural methods employed with *Aspergillus niger* have been given in previous publications (*13*) and need not be repeated except to state that the fungus was grown for 4 days at 35° C. in 200-cc flasks containing 50 cc of nutrient solution. Additional necessary information is furnished in connection with the experimental data.

Chemical determinations were made by means of the official methods for ash (*1, p. 336, par. 8*), nitrogen (*1, p. 137, par. 53*), phosphorus (*1, p. 130, par. 306*) magnesium (*1, p. 124, par. 12*), and sulphur (*1, p. 129, pars. 28–29*). Hicks' (*4*) method was used for potassium after preparation of the sample by the method of West (*15*).

Only single determinations were made for total nitrogen inasmuch as many of the samples (combined mycelial felts of duplicate cultures) provided very little material for analysis. The determinations listed in table 3, however, were made in duplicate with material grown in 2-liter flasks.

OPTIMUM NUTRIENT SOLUTIONS

The plan employed in this study is based on the use of optimum solutions containing the minimum quantities of inorganic constituents sufficient for maximum growth of the fungus with 50 cc of nutrient solution for 4 days at 35° C. These solutions were developed from suboptimal solutions by successively adding small increments of such components as had proved deficient in quantity in the preceding test, until the proper concentrations had been determined. The number of successive trials necessarily varied with each of the five solutions but usually more than four trials were required. Only when further addition of a small quantity of each component of the nutrient solution failed to bring about an increase in yield were the solutions considered suitable for studying the effects of a variation of nitrogen on yield. The reverse statement, that a slight decrease in any constituent will result in a diminished yield, is also true, of course. The composition of the nutrient solutions thus developed is given in table 1. The salt requirements of the fungus with variation in concentration of sucrose seem to exactly parallel the concentration of sucrose at these levels, if due allowance is made for deviations due to uncontrollable variations in impurities. The lesser need for minerals with ammonium chloride than with sodium nitrate is correlated, in all probability, with the higher acidities developed in cultures with the former as a consequence of relative accumulation of the chloride ion.

TABLE 1.—*Composition per liter of optimum nutrient solutions* [1]

Constituent	Composition of solution having as source of nitrogen—				
	NH_4NO_3			NH_4Cl	$NaNO_3$
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Sucrose	25.0	50.0	75.0	50.0	50.0
NH_4NO_3, NH_4Cl, or $NaNO_3$	.88 (.84)	1.90	3.05	2.54	4.04
K_2HPO_4	.17	.35	.60	.42 (.35)	.42 (.35)
$MgSO_4.7H_2O$	.10	.25	.53	.30 (.25)	.30 (.25)
Fe	.00008 (7)	.00020	.00036	.00020	.00024
Zn	.00011 (10)	.00020	.00032	.00020	.00030
Cu	.00001	.00005	.00009	.00005	.00008
Mn	.00001	.00002	.00005	.00002	.00005
Mo	.00001	.00002	.00003	.00002	.00006

[1] The values in parentheses are those actually used in the experiments of table 2, where they differed from those of the solutions used in determining the nitrogen curves and obtaining material for chemical analysis.

Table 2 shows growth data obtained with the five optimum solutions. The percentages of maximum yield with the trace elements indicate the extent of these elements present as impurities in the solutions. The yields obtained by increasing the individual constituents are a measure of their deficiency, if any, in these solutions. Even casual examination will disclose that these solutions are practically optimum for growth. The yields, moreover, are proportional to the quantity of sugar and independent of the source of nitrogen. The yields per unit quantity of sugar are corrected values and not those actually obtained. In the preparation of the solutions the total volume called for is usually exceeded slightly. Correction for the excess has been estimated experimentally to be equivalent to 0.002 times the excess in cubic centimeters per liter. This value in milligrams is added to the yield per culture if the nutrient solution was too dilute, and if, as occasionally happens, the solution was too concentrated, it is substracted.

The values for certain of the mineral constituents of the mycelial felts grown in the three optimum solutions with ammonium nitrate are given in table 3 for each of the three sucrose concentrations studied. Several interesting features appear. The percentage contents of the different constituents in the mycelial felts are, on the whole, unusually low (*9*) and vary but little in the three solutions. The deviation from the mean of the three experiments is less than ± 5.0 percent for ash, nitrogen, phosphorus, and sulphur; ± 7.9 percent for potassium, and ± 10.5 percent for magnesium. The accuracy with which yields could be determined with certainty was but slightly better, however. Practically no difference was found between the percentages of utilization of the nutrient components at the three sucrose levels. In other words the analytical results with the mycelial felts grown in the three optimum solutions were practically identical with respect to percentages of mineral constituents in the felts and the degree to which these constituents were absorbed. Variation in nonprotoplasmic mineral constituents resulting from passive absorption readily accounts for any slight differences that occur.

TABLE 2.—*Growth phenomena with Aspergillus niger when grown in optimum solutions for 4 days at 35° C.*

Component omitted from (−) or added in excess to (+) the nutrient solution	NH_4NO_3 (2.5 percent of sucrose)				NH_4NO_3 (5.0 percent of sucrose)				NH_4NO_3 (7.5 percent of sucrose)				NH_4Cl (5.0 percent of sucrose)				$NaNO_3$ (5.0 percent of sucrose)			
	Yield per 1.25 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [1]	Yield per 3.75 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [1]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
None	534.2	100.00	2.21	10, br	1,152.0	100.00	2.37	9, bl	1,658.6	100.00	2.76	10, bl	1,009.2	100.00	1.46	6, bl	1,002.8	100.00	2.81	10, bl
−Fe	104.6	19.58	2.25	1, bl	173.8	15.08	2.12	3, bl	322.2	19.43	1.98	4, bl	484.7	48.03	1.67	4, bl	207.3	20.67	3.25	8, bl
−Zn	35.5	6.64	2.76	2, bl	106.6	9.25	2.46	6, bl	172.7	10.41	2.33	4, bl	44.9	4.45	1.93	4, bl	211.1	21.05	3.16	8, bl
−Cu	504.9	94.51	3.06	4, t	1,011.6	87.81	3.15	1, bl	1,449.4	87.39	3.65	2, w	950.9	94.22	1.44	0	893.8	89.13	3.86	6, t
−Mn	431.7	80.85	1.82	0	899.7	78.10	1.68	1, bl	1,370.3	82.62	1.72	2, bl	778.5	77.14	1.35	1, bl	922.9	92.03	2.11	6, bl
−Mo	380.5	71.23	2.16	2, bl	706.3	61.30	1.82	1, bl	1,082.6	65.27	1.67	2, bl	1,012.2	100.30	1.42	3, bl	108.7	10.84	3.30	6, bl
+Fe	558.9	104.61	.....	10, br	1,203.0	104.42	.....	9, bl	1,646.0	98.07	.....	10, bl	984.9	105.60	.....	4, bl	1,013.5	101.08	.....	10, bl
+Zn	562.8	105.35	.....	10, br	1,162.6	100.92	.....	9, bl	1,630.2	97.13	.....	9, bl	988.8	97.98	.....	4, bl	1,038.1	103.53	.....	10, bl
+Cu	529.0	99.02	.....	10, bl	1,100.8	95.35	.....	9, bl	1,691.5	101.98	.....	10, bl	1,005.9	99.68	.....	4, bl	1,036.2	103.33	.....	10, bl
+Mn	535.2	100.18	.....	10, br	1,144.0	99.31	.....	9, bl	1,593.8	95.02	.....	9, bl	991.3	98.24	.....	4, bl	1,039.7	103.69	.....	10, bl
+Mo	542.7	101.58	.....	10, br	1,159.2	100.62	.....	9, bl	1,648.9	98.25	.....	9, bl	1,016.6	100.74	.....	4, bl	1,032.9	103.01	.....	10, bl
+N	562.9	105.37	.....	10, br	1,142.3	99.16	.....	4, bl	1,661.6	100.18	.....	9, bl	[2] 1,028.9	101.84	.....	2, bl	[2] 1,035.3	103.24	.....	10, bl
+K, P	520.2	97.44	.....	10, br	1,119.2	97.15	.....	9, bl	1,692.2	102.03	.....	10, bl	938.9	93.03	.....	4, bl	1,119.4	111.63	.....	10, bl
+Mg, S	537.0	100.53	.....	10, br	1,152.1	100.01	.....	9, bl	1,681.3	101.36	.....	10, bl	1,016.9	100.76	.....	4, bl	1,026.3	102.34	.....	10, bl
Max.[3]	562.9	.....	.....	.....	1,203.0	.....	.....	.....	1,692.2	.....	.....	.....	1,028.9	.....	.....	.....	1,119.4	.....	.....	.....
C. U.[4]	.....	45.03	.....	.....	.....	48.12	.....	.....	.....	45.12	.....	.....	.....	41.16	.....	.....	.....	44.78	.....	.....
pH [5]	.....	.....	7.13	.....	.....	.....	7.15	.....	.....	.....	7.18	.....	.....	.....	7.13	.....	.....	.....	7.45	.....

[1] Sporulation is indicated on a scale of 0 (none) to 10 (complete); and spore color by the initial letter or letters of the words black, brown, tan, and white.
[2] Increased acidity with sulphuric acid, without addition of nitrogen.
[3] Maximum individual yield.
[4] Coefficient of utilization or yield per 100 g of sucrose.
[5] Initial acidity of the nutrient solution.

TABLE 3.—*Mineral content of Aspergillus niger grown for 4 days at 35° C. in optimum solutions (NH_4NO_3) of different sucrose concentrations*

Constituent	Mineral content of organism with—								
	25 g of sucrose per liter			50 g of sucrose per liter			75 g of sucrose per liter		
	In mycelial felts dried at 103° C.	Quantity of [1] constituent—		In mycelial felts dried at 103° C.	Quantity of [1] constituent—		In mycelial felts dried at 103° C.	Quantity of [1] constituent—	
		Supplied [2] per liter	Absorbed by organism		Supplied [2] per liter	Absorbed by organism		Supplied [2] per liter	Absorbed by organism
	Percent	*Mg*	*Percent*	*Percent*	*Mg*	*Percent*	*Percent*	*Mg*	*Percent*
Ash	1.55	210.0	78.9	1.65	453.8	83.8	1.88	827.4	75.4
Nitrogen	2.62	308.0	90.9	2.83	665.0	98.0	2.98	1,067.5	92.6
Potassium	.61	76.3	85.1	.59	157.2	86.5	.60	269.4	73.9
Phosphorus	.26	30.3	91.7	.26	62.3	96.1	.31	106.8	96.3
Magnesium	.05	9.9	53.5	.05	24.8	46.4	.07	52.5	44.2
Sulphur	.08	13.0	65.8	.10	32.5	70.9	.13	68.9	62.6

[1] Computed on the basis of the quantities given in tables 1 and 2 for nutrient-solution constituents and yields of oven-dried tissue.

[2] The quantity of ash supplied is a computed value and does not include volatile constituents, such as ammonium nitrate, water of crystallization, and water lost through conversion of orthophosphate to pyrophosphate on ashing. The ash was quite deliquescent and free from carbonate.

NITROGEN GROWTH CURVES

The data showing the influence of the quantity of nitrogen on the growth of the fungus have been tabulated in table 4. These data will be discussed subsequently at greater length with the aid of graphic illustrations. The question of precision in results is best studied at this point, however.

The total number of pairs of duplicates is 120. Table 4 shows the deviation values for 119 pairs. Of these, 54, or 45 percent, deviated from the average by no more than ±1 percent; 81, or 68 percent, by no more than ±2 percent; 97, or 82 percent, by no more than ±3 percent; and 113, or 95 percent, by no more than ±5 percent. The maximum variation was ±9.1 percent. That this precision is subject to improvement is possible, since the procedures used were the ordinary methods, devised for speed as well as accuracy. It is, at any rate, definitely superior to the precision attainable in growth studies with green plants, as well as that formerly considered acceptable for work with *Aspergillus*. Ekman (*2*) studied the precision of his experimental data with this organism and found that of 130 duplicate determinations only 61 percent varied by no more than ±5 percent. Four of his duplicates varied by ±25 percent.

Reproducibility of results in successive experiments is of course the more important criterion of accuracy. The two experiments with varying nitrogen at a sucrose concentration of 7.5 percent afford material for a comparison, if the values in the second experiment are computed for nitrogen concentrations used in the first. They were performed a month apart. For the 19 values corresponding to those of the first determination, the deviations from the average were as follows: 5, or 26 percent of the total, vary by less than ±1 percent; 10, or 53 percent of the total, by less than ±2 percent; and 14, or 74 percent of the total, by less than ±3 percent. In none of the 19 cases did the variation exceed ±5 percent. Acidity values (pH), though quite uniform with the lower nitrogen concentrations, varied by as much as 0.5 of a unit at the higher concentrations. Increased experimental precision should give improvement in this respect also, but may be difficult to attain without a knowledge of all the essential elements.

TABLE 4.—*Yields of Aspergillus niger with graduated quantities of nitrogen when grown for 4 days at 35° C.*

2.5-percent sucrose solution+NH_4NO_3 (pH=7.16)						5.0-percent sucrose solution+NH_4NO_3 (pH=7.32)					
Nitrogen per liter	Individual cultures		Average	Deviation	Acidity	Nitrogen per liter	Individual cultures		Average	Deviation	Acidity
Mg	*Mg*	*Mg*	*Mg*	*Per cent*	*pH*	*Mg*	*Mg*	*Mg*	*Mg*	*Per cent*	*pH*
0	0.0	0.0	0.0	0.0	4.55	0	0.0	0.0	0.0	0.0	5.37
20	34.1	33.1	33.6	±1.5	2.71	40	66.9	73.6	70.3	±4.7	2.70
40	73.9	66.8	70.4	±5.1	2.66	80	153.0	146.1	149.6	±2.3	2.58
60	113.7	116.6	115.2	±1.2	2.64	120	238.1	235.0	236.6	±.7	2.56
80	153.4	141.8	147.6	±3.9	2.65	160	307.1	309.7	308.4	±.4	2.55
100	210.0	218.7	214.4	±2.0	2.63	200	408.1	399.5	403.8	±1.1	2.55
120	229.8	252.6	241.2	±4.7	2.70	240	466.0	497.2	481.6	±3.2	2.53
140	233.9	277.2	255.6	±8.4	2.64	280	552.4	546.6	549.5	±.5	2.46
160	284.7	290.2	287.5	±.9	2.55	320	605.9	617.4	611.7	±.9	2.39
180	304.7	311.4	308.1	±1.1	2.44	360	661.6	644.3	653.0	±1.3	2.32
200	328.3	358.8	343.6	±4.4	2.28	400	700.0	712.0	706.0	±.9	2.25
220	394.4	417.0	405.7	±2.8	2.33	440	796.9	803.9	800.4	±.4	2.28
240	405.4	440.6	423.0	±4.2	2.44	480	879.5	907.9	893.7	±1.6	2.38
260	493.3	486.2	489.8	±.8	2.58	520	989.6	958.2	973.9	±1.6	2.58
280	504.0	511.5	507.8	±.7	2.68	560	1,012.2	1,046.0	1,029.1	±1.6	2.70
300	536.7	507.4	522.1	±2.8	2.83	600	1,056.0	997.2	1,026.6	±2.9	2.86
320	549.7	542.0	545.9	±.7	3.13	640	1,079.0	1,001.3	1,040.2	±3.7	3.03
340	547.9	562.9	555.4	±1.4	2.57	680	1,099.1	1,109.5	1,104.3	±.5	2.86
360	551.6	562.0	556.8	±.9	2.41	720	1,114.8	1,131.3	1,123.1	±.7	2.75
380	539.4	554.2	546.8	±1.4	2.11	760	1,101.9	1,102.6	1,102.3	.0	2.47

7.5-percent sucrose solution+NH_4NO_3 (pH=7.38)						7.5-percent sucrose solution+NH_4NO_3 (pH=7.28)					
0	0.0	0.0	0.0	0.0	6.33	80	135.1	162.2	148.7	±9.1	2.65
60	115.9	113.0	114.5	±1.3	2.72	160	296.7	299.6	298.4	±.4	2.54
120	243.4	240.0	241.7	±.7	2.61	240	483.0	473.8	478.4	±1.0	2.50
180	370.8	386.2	378.5	±2.0	2.64	320	646.1	641.9	644.0	±.3	2.45
240	465.7	489.5	477.6	±2.5	2.54	400	764.7	755.7	760.2	±.6	2.40
300	581.6	622.0	601.8	±3.4	2.48	480	938.6	913.4	926.0	±1.4	2.35
360	716.4	747.6	732.0	±2.1	2.44	560	1,063.1	(1,211.0)	1,063.1	(±6.5)	2.39
420	754.2	834.0	794.1	±5.0	2.37	640	1,192.1	1,200.7	1,196.4	±.4	2.39
480	844.3	873.9	859.1	±1.7	2.33	720	1,333.6	1,269.0	1,301.3	±2.5	2.35
540	912.5	973.5	943.0	±3.2	2.28	800	1,444.7	1,376.6	1,410.7	±2.4	2.33
600	1,023.3	1,064.2	1,043.8	±2.0	2.24	880	1,474.8	1,500.6	1,487.7	±.9	2.29
660	1,180.7	1,208.4	1,194.6	±1.2	2.28	960	1,542.2	1,524.8	1,533.5	±.6	2.69
720	1,273.6	1,356.0	1,314.8	±3.1	2.34	1,040	1,602.5	1,574.4	1,588.5	±.9	2.95
780	1,442.2	1,438.8	1,440.5	±.1	2.55	1,120	1,612.8	1,613.2	1,613.0	.0	3.25
840	1,518.2	1,494.3	1,506.3	±.8	2.68	1,200	1,597.4	1,554.6	1,576.0	±1.4	3.24
900	1,531.9	(1,221.4)	1,531.9	?	3.03	1,280	1,565.8	1,546.1	1,556.0	±.6	2.71
960	1,550.5	1,503.9	1,527.2	±1.5	3.16	1,360	1,537.4	1,535.4	1,536.4	±.1	2.42
1,020	1,583.5	1,546.4	1,565.0	±1.2	3.32	1,440	1,580.2	1,506.9	1,543.6	±2.4	2.71
1,080	1,593.9	1,562.3	1,578.1	±1.0	3.47	1,520	1,550.1	1,547.1	1,548.6	±.1	2.44
1,140	1,604.3	1,586.3	1,595.3	±.6	2.99	1,600	1,576.6	1,587.1	1,581.9	±.3	2.08

5.0-percent sucrose solution+NH_4Cl (pH=7.44)						5.0-percent sucrose solution+$NaNO_3$ (pH=7.44)					
38	67.8	67.9	67.9	±0.1	2.74	34	53.0	55.7	54.4	±2.6	2.82
76	171.3	150.9	161.1	±6.3	2.46	68	123.7	122.7	123.2	±.4	2.72
114	226.7	231.7	229.2	±1.1	2.27	112	197.8	185.8	191.8	±.3	2.71
152	276.5	302.3	289.4	±4.5	2.14	146	247.2	234.8	241.0	±2.6	2.71
190	357.5	345.1	351.3	±1.8	2.03	180	310.8	318.6	314.7	±1.2	2.76
228	416.5	442.4	429.5	±3.0	1.93	214	372.8	367.4	370.1	±.7	2.76
266	456.0	473.2	469.6	±.8	1.88	248	379.7	399.7	389.7	±2.6	2.81
304	534.6	539.8	537.2	±.5	1.81	282	442.6	463.0	452.8	±2.3	2.83
342	595.6	596.0	595.8	.0	1.75	316	473.9	477.0	475.5	±.3	2.86
380	672.7	657.6	665.2	±1.1	1.68	350	542.6	551.3	547.0	±.8	2.84
418	706.0	784.9	745.5	±5.3	1.61	384	584.6	561.3	573.0	±2.1	2.62
456	913.4	851.0	882.2	±3.5	1.60	418	628.8	596.8	612.8	±.3	2.36
494	866.3	857.4	861.9	±.5	1.55	452	636.8	679.6	658.2	±3.3	2.03
532	901.4	974.9	938.2	±3.9	1.53	486	701.8	685.1	693.5	±1.4	2.21
570	845.2	854.5	849.9	±.6	1.53	520	774.3	775.7	775.0	±.1	2.08
608	990.8	978.4	984.6	±.6	1.48	554	793.6	737.0	765.3	±3.7	2.03
646	1,028.5	991.1	1,009.8	±1.9	1.41	588	823.8	824.9	824.4	±.1	2.08
684	1,010.7	1,019.0	1,014.9	±.4	1.42	622	857.4	846.8	852.1	±.6	2.05
722	1,050.7	1,004.1	1,027.4	±2.3	1.42	656	895.5	885.9	890.7	±.5	2.05
760	1,074.0	1,064.1	1,069.1	±.5	1.39	690	942.3	920.3	931.3	±1.2	2.08

NITROGEN PARTITION IN THE CULTURES

The nitrogen content (total) of the mycelial felts grown with ammonium nitrate at the three levels of carbohydrate nutrition has been summarized in table 5. The percentage of nitrogen in the dried tissues of the organism is fairly uniform for analogous concentrations. It is slightly lower at the lower nitrogen levels and increases very gradually until the optima for growth are reached. Whether the slight increases in the percentage of nitrogen at the optima when sugar is increased be of significance is problematical. Comparisons between the nitrogen utilized by the organism at identical levels would indicate that nitrogen was the limiting factor for growth, since the amount of nitrogen absorbed was influenced but little by the concentration of the other constituents present. For example, the amount of nitrogen removed from the solution at the 360-mg level with 2.5 percent of sucrose was 15.4 mg; with 5.0 percent of sucrose, 16.0 mg; and with 7.5 percent of sucrose, 14.0 mg.

TABLE 5.—*Distribution of nitrogen in cultures of Aspergillus niger with graduated quantities of nitrogen when grown for 4 days at 35° C.*

2.5 percent of sucrose					5.0 percent of sucrose					7.5 percent of sucrose				
Nitrogen per liter	Nitrogen in mycelium			Nitrogen used	Nitrogen per liter	Nitrogen in mycelium			Nitrogen used	Nitrogen per liter	Nitrogen in mycelium			Nitrogen used
	Found	Calculated	Per culture			Found	Calculated	Per culture			Found	Calculated	Per culture	
Mg	*Per cent*	*Per cent*	*Mg*	*Per cent*	*Mg*	*Per cent*	*Per cent*	*Mg*	*Per cent*	*Mg*	*Per cent*	*Per cent*	*Mg*	*Per cent*
20	2.169	2.04	0.7	73.0	40	2.063	2.03	1.5	72.5	60	1.923	2.03	2.2	73.4
40	1.981	2.06	1.4	69.5	80	2.030	2.05	3.0	75.9	120	2.035	2.04	4.9	81.7
60	2.033	2.07	2.3	78.0	120	2.072	2.07	4.9	81.7	180	2.035	2.05	7.7	87.8
80	2.055	2.13	3.0	75.8	160	2.109	2.10	6.5	81.3	240	1.742	2.09	8.3	69.3
100	1.819	2.08	3.9	78.0	200	2.100	2.10	8.5	84.8	300	1.622	2.11	9.8	65.1
120	2.105	2.13	5.1	84.7	240	2.190	2.13	10.6	87.8	360	1.834	2.13	13.4	74.6
140	2.350	2.20	6.0	86.0	280	2.277	2.16	12.4	88.7	420	1.988	2.19	15.6	74.3
160	2.392	2.24	6.9	86.0	320	2.338	2.21	14.3	89.4	480	2.100	2.26	18.0	75.2
180	2.429	2.31	7.5	83.1	360	2.448	2.25	16.0	88.8	540	2.157	2.31	20.3	75.3
200	2.321	2.34	8.7	86.6	400	2.532	2.31	17.9	89.4	600	2.297	2.34	24.0	79.9
220	2.348	2.31	9.5	86.6	440	2.213	2.31	17.7	80.5	660	2.353	2.34	28.1	85.2
240	2.456	2.38	10.4	86.6	480	2.213	2.33	19.8	82.4	720	2.381	2.36	31.1	86.9
260	2.296	2.35	11.3	86.5	520	2.269	2.30	22.1	85.0	780	2.283	2.38	32.9	84.3
280	2.383	2.41	12.1	86.4	560	2.367	2.40	24.4	87.0	840	2.381	2.45	35.9	85.4
300	2.538	2.49	13.3	88.3	600	2.494	2.51	25.6	85.3	900	2.760	2.54	42.3	94.0
320	2.482	2.55	13.6	84.7	640	2.634	2.60	27.4	85.6	960	2.718	2.66	41.5	86.4
340	2.632	2.64	14.6	86.0	680	2.620	2.64	28.9	85.1	1,020	2.971	2.74	46.5	91.2
360	2.757	2.74	15.4	85.3	720	2.760	2.72	31.0	86.1	1,080	3.097	2.84	48.9	90.5
380	2.842	2.87	15.5	81.8	760	2.971	2.80	32.8	86.2	1,140	3.069	2.94	49.0	85.9

7.5 percent of sucrose					7.5 percent of sucrose (adjusted values)				7.5 percent of sucrose (average)			
80	2.019	2.04	3.0	75.1	60	2.005	2.4	74.4	60	1.964	2.3	73.9
160	2.076	2.08	6.2	77.8	120	2.048	4.6	76.5	120	2.042	4.8	79.1
240	2.112	2.09	10.1	84.2	180	2.085	7.2	79.4	180	2.060	7.4	83.6
320	2.075	2.12	13.4	83.5	240	2.112	10.1	84.2	240	1.927	9.2	76.8
400	2.058	2.18	15.6	78.2	300	2.084	12.6	83.6	300	1.853	11.2	74.4
480	2.058	2.20	19.1	77.6	360	2.067	14.5	80.9	360	1.951	14.0	77.8
560	2.086	2.25	22.2	75.6	420	2.058	16.5	78.0	420	2.023	16.1	76.2
640	2.255	2.30	27.0	84.3	480	2.058	19.1	77.6	480	2.079	18.6	76.4
720	2.297	2.34	29.9	83.0	540	2.079	21.4	78.1	540	2.118	20.9	78.7
800	2.381	2.44	33.6	84.0	600	2.171	24.6	80.0	600	2.234	24.3	80.0
880	2.494	2.52	37.1	84.3	660	2.266	27.7	84.0	660	2.310	27.9	84.6
960	2.662	2.64	40.8	85.0	720	2.297	29.9	83.0	720	2.339	30.6	85.0
1,040	2.746	2.76	43.6	83.9	780	2.360	32.7	83.8	780	2.325	32.8	84.1
1,120	3.041	2.88	49.1	87.6	840	2.438	35.4	84.2	840	2.410	35.6	84.8
1,200	3.294	3.07	51.9	86.5	900	2.536	38.0	84.5	900	2.648	40.2	89.3
1,280	3.490	3.23	54.3	84.9	960	2.662	40.8	85.0	960	2.690	41.2	85.7
1,360	3.462	3.38	53.2	78.2	1,020	2.725	42.9	84.1	1,020	2.848	44.7	87.7
1,440	3.673	3.53	56.7	78.7	1,080	2.894	46.3	85.8	1,080	2.996	47.6	88.2
1,520	3.771	3.67	58.4	76.8	1,140	3.103	49.8	87.3	1,140	3.086	49.4	86.6
1,600	3.869	3.81	61.2	76.5								

Computation of the percentages of nitrogen not absorbed gave values of much interest that were not anticipated. Intake seemed to be at a minimum with minimum nitrogen supply. Even at concentrations about 5 percent of optimum only about 75 percent of the nitrogen was utilized. Percentages of nitrogen absorbed are in accordance, nevertheless, with the observation of Roberg (*10*) that this fungus cannot remove iron completely from solution even at extreme deficiency levels.

The data of table 5 also afford a basis for an interesting analysis with respect to total nitrogen. If the assumption is made that protoplasmic nitrogen in the mycelial felts is fairly constant, the excess nitrogen in the mycelial felts must be absorbed nitrogen that has not become an integral part of the protoplasm. The fungus mycelium is, of course, in close contact with its nutrient solution and probably does not store nitrogen in any form. The partition of the nonprotoplasmic nitrogen in the culture, if similar to that of a compound between two immiscible solvents, would be proportional to the coefficient of distribution and the relative quantities of felt and solution in the protoplast-nutrient solution system. Calculations for total nitrogen in the dry mycelial felts were made on this basis for each chemical determination of total nitrogen. Nonprotoplasmic nitrogen was taken to equal the difference between the amount added and the 2-percent content of the mycelia under starvation conditions. Of this quantity, $0.6 \frac{\text{yield}}{\text{maximum yield}} \times$ nonprotoplasmic nitrogen equals the nitrogen present passively in the organism. The constant equaling 0.6 corresponds to a coefficient of distribution of 1.5, and implies that the ammonium nitrate was one and one-half times more soluble in the protoplast than in the solution. The computed percentages of total nitrogen in the mycelial felts agree with the determined values within the limits of experimental and chemical precision. The values for found and calculated percentages of total nitrogen did not differ by more than approximately 0.1 percent of total nitrogen in 48 out of 77 determinations (62 percent). Sixty-six, or 86 percent, agreed within 0.2 percent of total nitrogen. Moreover, the 11 remaining values were just those representing determinations that seemed irregular. If not a proof of the correctness of the above premises, it must, nevertheless, be granted that they afford an empirical means for the accurate computation of total nitrogen under these experimental conditions.

RELATIVE YIELD AND NITROGEN CONTENT AT DIFFERENT SUCROSE LEVELS

Superimposition of the corresponding curves representing yield and nitrogen absorbed for the three levels of carbohydrate nutrition was brought about in figure 1 by multiplying all values by 2 at the 2.5-percent level, by 1 at the 5.0-percent level, and by two-thirds at the 7.5-percent level. There would seem to be little doubt that a high degree of coincidence exists, especially if due allowance be made for probable errors in the determination of the values. The yield curves are similar in form to those frequently reported but show definite deviations from uniformity. All four curves with ammonium nitrate are slightly concave to the X-axis at minimum concentrations,

and slightly convex at moderate concentrations. The graphs for nitrogen utilization seem to be linear.

Figure 2 gives a comparison of the yields obtained in a 5.0-percent sucrose solution with ammonium nitrogen, with nitrate nitrogen, and with equal amounts of each (NH_4NO_3). Though maximum yields are the same with all these sources of nitrogen (*13*), the intermediate values differ appreciably. Ammonium nitrate gave the highest intermediate yields, sodium nitrate the lowest intermediate yields, while ammonium chloride gave median yields. These differences in yield would seem to be correlated with the acidities of the nutrient solution, especially if it is assumed that the use of ammonium chloride

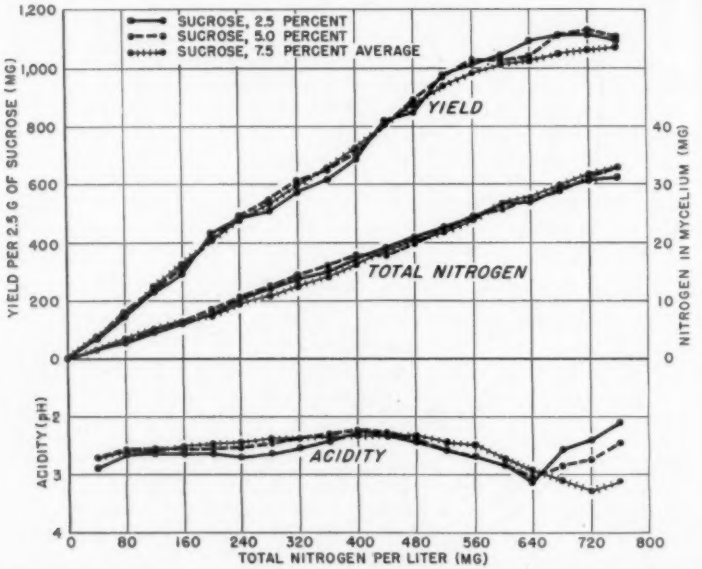

FIGURE 1.—Superimposed curves for yield, total nitrogen content, and acidities at harvest when graduated quantities of nitrogen as ammonium nitrate were used with *Aspergillus niger* grown for 4 days at 35° C. Superimposition was brought about by multiplying all values, except acidity, by 2 for 2.5 percent of sucrose, by 1 for 5.0 percent of sucrose, and by two-thirds for 7.5 percent of sucrose.

results in excessive acidities at intermediate nitrogen-deficiency levels.

The acidities of the culture solutions at harvest are of course the resultant of residual electrolytes and organic acids synthesized by the fungus. The favorable effects of ammonium nitrate, if attributable to acidity, might follow through the selective utilization of the ammonium and nitrate ions.

THEORETICAL GROWTH CURVE FOR NITROGEN

Without doubt the growth formula suggested by Mitscherlich (*11*) has aroused most discussion and led to more work than any other. Mitscherlich claimed that the constants of his equation have a definite

biological meaning and that the value of c in the formula $y=A(1-10^{-cx})$ is dependent only on the type of fertilizer or growth factor. The value A represents the maximum yield, and y the yield with a quantity (x) of nitrogen, phosphorus, etc. Mitscherlich (*11*), despite his claims for the theoretical accuracy of his formula, later found it necessary to modify the above expression to $y=A(1-10^{-cx})\ 10^{-kx^2}$ in order to make his formula suitable for computing negative increments in yield with excess fertilizer. Extensive data have been

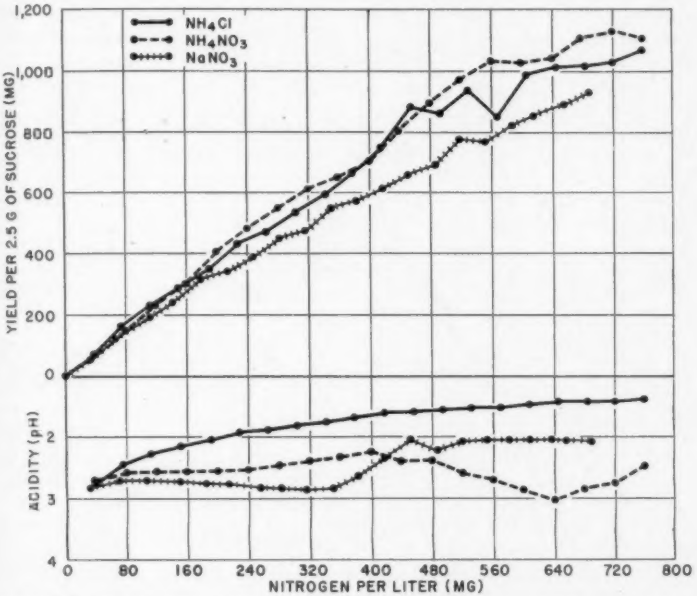

FIGURE 2.—Yield and acidity curves when graduated quantities of nitrogen, supplied as ammonium chloride, ammonium nitrate, and sodium nitrate were used with *Aspergillus niger* grown for 4 days at 35° C. The sucrose level was 5 percent.

accumulated by Meyer (*6*) with *Aspergillus niger* for use in the mathematical analysis of the Mitscherlich formula.

A brief study of the data herein presented has been made in this connection and is represented by the graphs of figure 3. Curves *a* and *b* are replicate yield curves for ammonium nitrate and 7.5 percent of sucrose, obtained a month apart. Their values are averaged in curve *c*. The original Mitscherlich equation is represented by curve *d*, the value for the maximum yield (A=4,837.6) being computed for nitrogen by his method. Curves *e* and *f* are also Mitscherlich yield graphs computed for the maximum yield (1,600) obtained experimentally. The remaining curve (*g*) represents an empirical equation sometimes used to express engineering data.

It is evident that none of the computed curves agree with the experimental growth curve. The Mitscherlich equation of growth can be made to agree quite closely with the lower yield values (*d*) or

the maximum value (*e*, *f*), but it cannot be made to give figures in agreement with all values of the experimental curve. Use of the modified Mitscherlich formula gave results but little better than

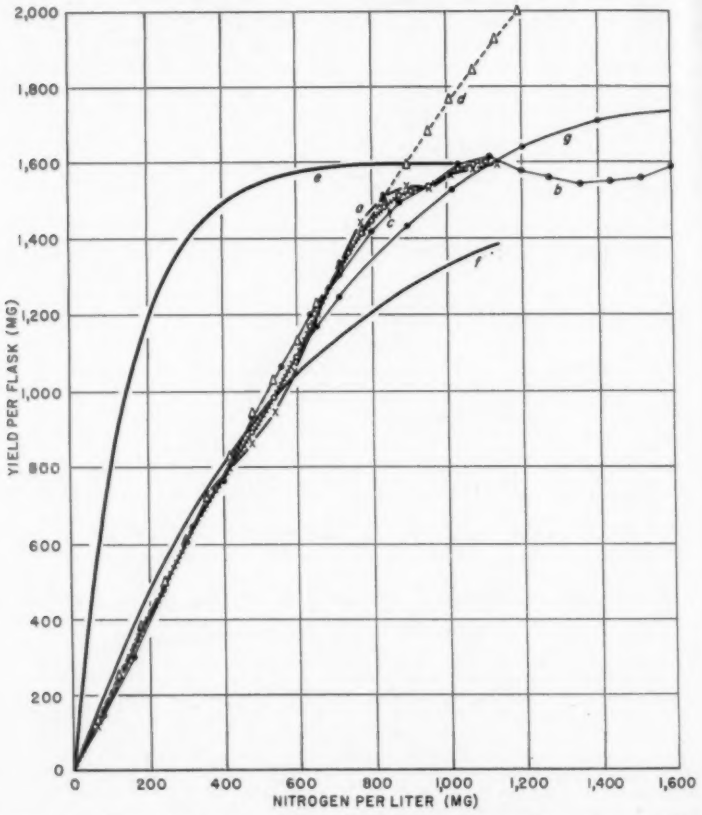

FIGURE 3.—Computed and experimental yields when graduated quantities of nitrogen were used with *Aspergillus niger* grown for 4 days at 35° C. in an optimum solution containing 7.5 percent of sucrose. Curves *a* and *b* are replicate determinations, and curve *c* is their average. Curves *d*, *e*, and *f* were computed with the Mitscherlich growth formula $y=A\ (1-10^{-cx})$. In curve *d*, *A* equals 4,837.6 and *c* is 0.0001927. The constants for curve *e* are $A=1{,}600$ and $c=0.0028$; and for curve *f*, $A=1{,}600$ and $c=0.000761$. The constants for the empirical equation of curve *g* are, in formula form, $y-1{,}725=-0.0001493\ (1{,}632.5-x)^{2.2}$.

those of the empirical curve (*g*) for equation $y=a-b\ (c-x)^n$, in which y represents yield, x the nitrogen supply, and a, b, c, and n are constants.

DISCUSSION

Variation of nitrogen supply in optimum solutions at the three levels of sugar nutrition led to what are essentially identical results.

Graduated quantities of this element ranging from 0 to optimum gave in all cases yield curves of exponentiallike form and total-nitrogen curves of linear form that could be mutually superimposed quite closely. The yields and the quantities of nitrogen absorbed at all deficiency levels were nearly exactly proportional to the quantity supplied irrespective of the other constituents. In other words, the variations relative to the respective maxima were identical, and the maxima therefore are satisfactory for use as fixed physiological standards of reference. Later replication of these standard solutions, moreover, is readily possible with a satisfactory degree of accuracy, if the relative salt concentrations (1 : 2 : 3) required by the three optimum solutions with ammonium nitrate and relative yields furnish a criterion.

It was found possible to compute total nitrogen in the felts at nitrogen levels up to optimum, with a precision equal to that of the chemical determinations. The assumptions on which these calculations are based afford, therefore, a possible explanation of certain phases of nitrogen absorption and content of this organism. That is to say, the total nitrogen in *Aspergillus niger* is probably composed of a relatively fixed percentage of protoplasmic nitrogen and a variable proportion of absorbed nutritive nitrogen. The quantity of the latter component in the felts is dependent on the concentration of nonprotoplasmic nitrogen available. Its distribution between organism and solution seems to be entirely in accordance with the physicochemical laws governing distribution of dissolved substances between immiscible solvents.

It is doubtful, however, that the results on absorption could hold for green plants in view of the morphological differences that exist. Their lack of direct and practically complete contact with the nutrient solution and their capacity for nitrogen storage have indeed led to results of a different character (*5*, *7*). Nevertheless, these results render it possible that biological absorption in its simplest form may be entirely passive, even for negative protoplast-solution gradients, and based on the relative solubilities of a substance in the protoplast and solution. The use of a nutrient in concentrations higher than optimum may be expected to lead to changes in the protoplasm that will materially alter these responses. Other additions may be equally effective in this respect. Recognition of these factors does not vitiate the importance of the type of absorption due wholly to solubility, but offers a point of departure in the study of the influence of these factors on absorption.

Though the interpretation of these results on the basis of absorption has been emphasized because of its importance, if correct, to an understanding of these phenomena, it is by no means the only possible explanation. No data are available on the form and location of the nitrogen in the tissues. The appearance, quantity, and composition of the cell-wall material of the hyphae undergo a regular change within the 0-to-optimum nitrogen range. Since nitrogen enters into the composition of the cell wall of this fungus, the importance of obtaining additional information before coming to a final decision can be realized. Despite the fact, however, that either or both of these views may be right, the regularity of the changes in composition and their ease of duplication would warrant additional investigation.

The yield curves with ammonium and nitrate nitrogen afford a concrete illustration of frequent error in quantitative comparisons of utility with nutrients. The ratios in yield with these two sources of nitrogen vary throughout the whole range, even though the optimum nitrogen concentrations and the maximum yields for both are identical (*13*). That even more striking differences may occur in the absence of proper concentrations of the trace elements has been demonstrated in the paper just cited. Prior knowledge of the physiological effect of each constituent and of its concentration is a necessary requirement, therefore, for the interpretation of studies in nutrient efficiency.

The quantitative concordance in relative results for yields, acidity, nitrogen absorbed, and the percentage of nitrogen used in the three optimum solutions when the nitrogen supply was varied is considered to be a demonstration of the suitability of results with an optimum solution of minimum salt content as biological reference standard. The constancy in mineral contents of the fungus and in the percentages at which they were utilized furnish additional evidence to strengthen this interpretation. Ordinarily a variation in supply of one mineral constituent results in a variation in the percentages of the others found in the tissues. Many instances of this type of behavior have been determined by Rennerfelt (*9*) at a concentration range of 1 to 100. Employment by this investigator of an optimum solution of minimum salt content would, it is believed, have led to different results in certain instances. With the three optimum solutions having sucrose contents as 1, 2, 3, the mineral requirements and the yields bore the same proportions. The mineral composition of the fungus felts grown on these solutions and the percentages of mineral utilization were identical, within experimental precision, in all three instances.

These results also lead to the impression that the formula advocated by Mitscherlich (*11*), though showing close agreement with certain portions of the nitrogen growth curve of *Aspergillus niger*, has no general theoretical significance. Even the new formula is but slightly better for representing yields than a purely empirical equation selected almost at random. Furthermore the growth curve for nitrogen (NH_4NO_3) is not uniform but has several points of inflection that do not lend themselves to mathematical expression with the comparatively simple formulations so far proposed. The computed value of A, the maximum yield, is, for nitrogen at least, of no biological significance with *Aspergillus*. The view of Mitscherlich (*11*) that the maximum yield for nitrogen can never be obtained in practice but must be computed fails to be convincing when it necessitates assuming that *A. niger*, with a total quantity of 3,750 mg of sucrose per culture, should be capable of giving a maximum yield of 4,838 mg. Actually, of course, the situation is that the Mitscherlich curve may be computed to agree with many of the lower values sufficiently for practical purposes but cannot be computed to agree with all the experimental yields in one and the same curve. A similar situation was found to exist by Garner et al. (*3*) with tobacco. In substance, rejection of the experimentally determined nitrogen maximum amounts to rejection of the experimental criteria for accuracy of the calculated yields in order to eliminate a disagreement in results.

There can be little doubt that the points of inflection found in the nitrogen growth curve for *Aspergillus* have an actual existence. They

occur in every experimental curve with ammonium nitrate as the source of nitrogen, even though the sugar concentration varied as 1 to 3, and may be associated with changes in the relative rates of absorption of ammonium and nitrate ion. Yields with ammonium chloride and with sodium nitrate do not show similar divergencies. The relative positions of these growth curves for *Aspergillus* are the same as that found by Garner et al. (*3*) for the tobacco plant.

A comprehensive analysis of the agreement between found and computed yields at all deficiency levels cannot be made, however. Though the variation between duplicate yields and between replicate determinations is only ±5 percent or less, this accuracy is insufficient for the proper evaluation of the slight differences that exist for the lower values. At least double the precision is necessary, i. e., a variation of less than ±2 percent in replicate trials. Experimental determinations within this margin of error should be obtainable when all the essential elements have been identified and procedures are based solely on precision in results.

SUMMARY

Yield curves with graduated quantities of nitrogen were obtained with *Aspergillus niger* grown in optimum nutrient solutions for 4 days at 35° C. Forty-five percent of the duplicate cultures agreed to within ±1 percent, 68 percent to within ±2 percent, 82 percent to within ±3 percent, and 95 percent to within ±5 percent. Yields could be verified to within ±5 percent on repetition. Growth with ammonium nitrate was studied at three levels of carbohydrate nutrition and was compared with growth obtained with ammonium chloride and with sodium nitrate. The growth and total-nitrogen curves obtained with 2.5, 5.0, and 7.5 percent of sucrose were found to be proportional and identical in form when superimposed. Other results for the three solutions were also practically identical. At intermediate nitrogen concentrations, ammonium chloride gave lower and sodium nitrate still lower yields than did ammonium nitrate. Total nitrogen in the mycelial felts grown with ammonium nitrate increased linearly with increasing concentrations of nitrogen. Values for total nitrogen in the felts could be computed within the limits of accuracy for the chemical determinations. The assumption was made that the fungus invariably contained 2 percent of protoplasmic nitrogen and that the balance of nitrogen supplied was partitioned between the organism and the solution similarly to the distribution of a dissolved substance between two immiscible solvents. The values of yield curves with ammonium nitrate could not be duplicated by computation with the Mitscherlich equation of growth.

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INFLUENCE OF VARIOUS FACTORS ON THE SHAPE OF BEET ROOTS[1]

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INTRODUCTION

To one acquainted with varieties of table beets (*Beta vulgaris* L.) no close examination is necessary to observe that individual roots within a variety represent many distinct types of root shape. On account of the difficulties involved in producing inbred beet seed, very few commercial strains are highly uniform for any morphological character. The great variation in shape of roots within a single strain raises the question as to its cause; doubtless in many cases it is due to genetic factors. The present studies were undertaken to determine whether certain environmental factors, including soil texture, soil fertility, and seasonal conditions correlated with time of planting, induce variations in the shape of beet roots that can be determined by shape indices.

Numerous investigations have been reported in which the shapes of various plant organs have been described in terms of numerical indices, including studies of the variation in shape of roots in some of the root crops. Edmond (*3*)[3] found that the depth to which the seeds were covered in planting had a measurable effect on the shape of radish roots. Mack and Lachman (*6*) presented data to show that consistent root-shape differences between beet varieties can be shown by numerical indices. Schermerhorn (*7*), Zimmerley (*9*), Carolus (*2*), and Boswell and Beattie (*1*) reported on the influence of different fertilizer treatments on the shape of sweetpotato roots as determined by an index obtained by comparing the polar and equatorial diameters. Pino[4] found that the shape of beet roots was influenced by the proportion that developed above and below the soil surface, and Hummel (*5*) worked out a comprehensive system of recording and interpolating root shapes.

PROCEDURE

PLANTINGS

Studies on root shape were confined to one strain of the beet variety Detroit Dark Red. In order to eliminate as far as possible variation due to genetic factors, a strain[5] was selected that was

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[2] The writer wishes to acknowledge his indebtedness to V. R. Boswell, of the Division of Fruit and Vegetable Crops and Diseases, for suggesting the problem and for helpful criticism throughout the investigations, and to Lester E. Barrett and William F. Kosar, of the same Division, for valuable assistance in recording the data and computing the indices.

[3] Italic numbers in parentheses refer to Literature Cited, p. 745.

[4] PINO, A. L. A STUDY OF THE TABLE BEET IN AN ATTEMPT TO DISCOVER THE CONDITIONS, CLIMATIC AND CULTURAL, WHICH CAUSE ITS DISCOLORATION. Unpublished thesis, Mich. State Col. Agr. and Appl. Sci. East Lansing, Mich. 1928.

[5] One of the most uniform strains in root shape in the vegetable standardization trials of the Division of Fruit and Vegetable Crops and Diseases.

known to produce roots of fairly uniform shape. The same strain was used throughout the investigations.

Root samples for these studies were harvested from 17 crops of beets grown over a period of 5 years; 6 of these crops were grown at Arlington Experiment Farm, Arlington, Va., and 11 at the United States Horticultural Station, Beltsville, Md. (table 1).

TABLE 1.—*Field record with planting and harvesting dates for the 17 crops of beets studied*

Location	Soil type	Moisture	Date planted	Date harvested
Arlington, Va.	Medium loam	Overhead irrigation	Mar. 19, 1933	June 8, 1933
			Apr. 27, 1933	Aug. 11, 1933
			Apr. 9, 1934	July 3, 1934
			May 9, 1934	Aug. 14, 1934
			June 7, 1934	Aug. 24, 1934
			Aug. 1, 1934	Oct. 12, 1934
Beltsville, Md.	Deep sand to clay loam	Natural precipitation	May 2, 1933	Aug. 2, 1933
			Apr. 12, 1934	June 26, 1934
			June 6, 1934	Aug. 7, 1934
Do.	Medium loam	Overhead irrigation	May 8, 1935	July 2, 1935
			June 4, 1935	Aug. 6, 1935
			July 9, 1935	Sept. 15, 1935
			Aug. 5, 1935	Oct. 11, 1935
			June 19, 1937	Aug. 23, 1937
			July 19, 1937	Sept. 28, 1937
			July 30, 1937	Sept. 30, 1937
			Aug. 23, 1937	Nov. 5, 1937

The six crops grown at Arlington Farm were on a medium loam soil of good fertility, ranging in pH value from 5.6 to 6.2. In both years the land was spring-plowed and fertilized at the rate of 1,500 pounds of 5–8–5 fertilizer per acre. The plots were provided with overhead irrigation and none was permitted to suffer for want of moisture.

The three crops grown at Beltsville in 1933 and 1934 were on land not equipped for irrigation and some were grown with a very limited moisture supply. The field used at Beltsville in 1933 and 1934 was long and narrow, having a soil gradient ranging from deep, loose sand at one end to heavy, reddish clay at the other. This area was comparatively low in fertility previous to the treatments given the fertilizer plots. Three treatments were used and each was duplicated. The plots receiving treatment 1 were given an application of well-decomposed stable manure at the rate of 10 tons per acre, supplemented with a 5–8–5 commercial fertilizer at the rate of 500 pounds per acre. The plots in treatment 2 received the 5–8–5 fertilizer at the rate of 1 ton per acre. In treatment 3 the plots were given an application of 500 pounds of 5–8–5 fertilizer. The plots were arranged in the field so that light sandy soil and heavy clay soil were included in each fertilizer treatment. The reaction of this soil ranged from pH 5.8 to 6.3.

The eight crops grown at Beltsville in 1935 and 1937 were produced on a medium loam soil of high fertility, having a pH range of 5.6 to 6.3. The soil was spring-plowed and fertilized with 5–8–5 fertilizer at the rate of 1,500 pounds per acre. This land was provided with overhead irrigation.

All of the 17 crops were grown in rows 18 inches apart. The planting was done with a mechanical seeder set to cover the seed to a depth of approximately three-quarters of an inch. When the seedlings had reached a height of about 2 inches they were thinned to a distance of 2 to 3 inches between plants in the row.

SAMPLING

In order to determine the number of roots required for a representative sample of a population, a study was made of the variability of the mean index values of samples consisting of 50, 100, and 200 roots. Root samples from three different populations were studied. The mean indices for 50-root samples were quite variable; those for the 100-root samples were much less so, and the differences in most cases were not significant. Very little reduction in variability in mean indices was obtained by increasing the sample from 100 to 200 roots. A random sample of 100 roots was therefore considered representative of a population.

The first data recorded were taken on samples of 100 roots, harvested at random, from the various populations to be studied. No attention was paid to the variation in size of the beets within the sample. When these data were analyzed it was found that the size of the root as indicated by its greatest equatorial width was a factor in determining the various shape indices. A large number of measurements were made to determine the influence of size on shape indices, the data for which will be presented later. It was necessary then to alter the method of taking samples so as to eliminate the variation due to size. All samples compared were of the same equatorial diameter.

RECORDING OF SHAPE DATA

After the samples had been harvested, washed, and dried, the top part was removed by a transverse cut at the point of the oldest leaf scar. A part of the lower end was removed by a transverse cut at a point about 2 cm. below the thickened portion. The roots were then halved longitudinally through a plane at right angles to the lateral rootlets. The outline of the cut surface was found to vary greatly, depending on the plane through which the cut was made. In order to obtain comparable data the longitudinal cut was made as nearly as possible through the same plane in relation to the lateral rootlets.

A permanent record of the shape of the individual roots was made by placing the cut surface of each root on a piece of white paper and carefully tracing the outline. This rapid method made it possible to record the shape of a much larger number of roots than could have been handled if all of the measurements had been made from the roots. From these records the necessary measurements were made.

MEASUREMENTS

The following measurements were taken for each root studied:

(1) The equatorial diameter (*d*) was taken at the point of greatest width as indicated by the line *bc* (fig. 1, *A*).

(2) The thickness (*ld*) of the root was taken at the point of greatest curvature at the base of the thickened portion.

(3) The polar diameter (*a*) was taken as the distance between the lines *ls* and *ld*, at the top and bottom, respectively, of the thickened portion of the root. The line *ls* was determined by the cut made in removing the top at the point of the oldest leaf scar.

(4) Measurements were made of the two portions of the polar diameter (*a*) resulting from its intersection with the equatorial diameter (*d*). The portion above the line *bc* was indicated by the letter *u* and the portion below the line by the letter *m* (fig. 1, *A*).

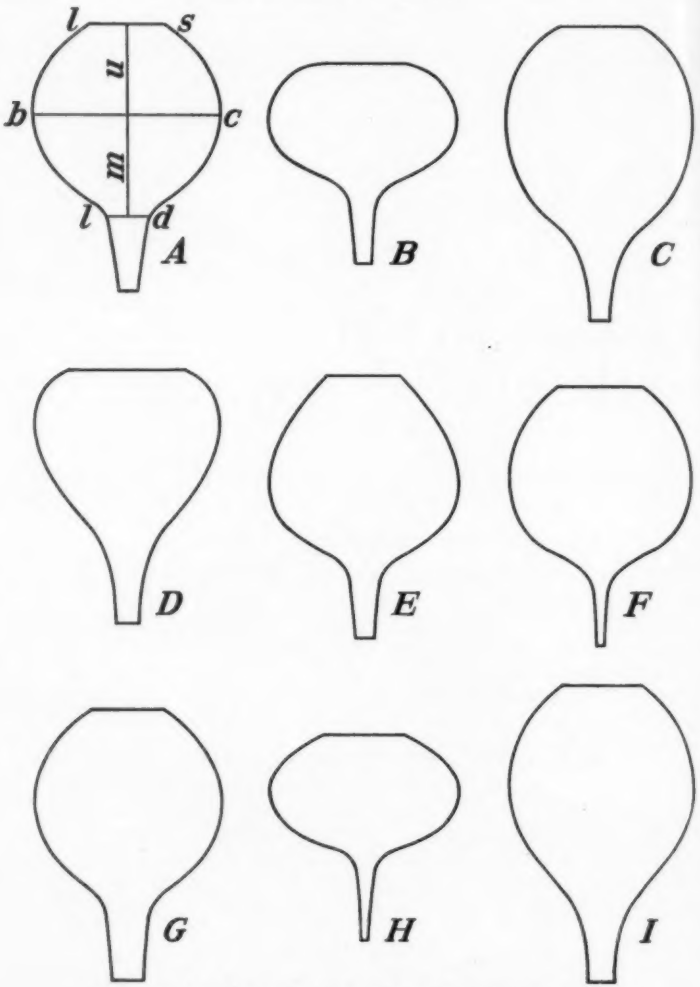

FIGURE 1.—FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

Botanically, the underground portion of a beet is largely a thickened taproot. In order to distinguish the thickened upper portion of the beet root from the lower, less thickened portion, the term "taproot" will be used to designate only the lower, unthickened portion below the line *ld*, determined by the point of greatest curvature.

INDICES

From the measurements described in the preceding section, three indices of shape were determined, namely, a/d, u/m, and ld/d.

An index of length (a/d) was derived by dividing the polar diameter (a) by the equatorial diameter (d). A value less than 1.00 for a/d indicates a flattened root; a root having an a/d index of 0.650 is shown in figure 1, *B*. A high a/d index indicates a long root; a root having an index of 1.200 is shown in figure 1, *C*. As the a/d index approaches unity, the root is more nearly spherical (fig. 1, *A*).

A ratio between the polar and equatorial diameters is probably the best single index of shape; however, great variations in shape may occur in beet roots having the same a/d index, some of which are shown in the outline drawings *D*, *E*, *F*, and *G* of figure 1. The variation in shape in *D* and *E* is due to the position of the intersection of the equatorial diameter (d) with the polar diameter (a). In order to study this shape variation, an index was derived by dividing the upper portion of the polar diameter (u) by the lower portion (m). A value less than 1.00 for the u/m index indicates a top-shaped root in which the greatest bulge is high on the polar diameter. A root of this type, having a u/m index of 0.400, is shown in figure 1, *D*. A value greater than 1.00 for u/m indicates a root having its greatest bulge low on the polar diameter; that is, with an inverted top-shaped appearance. A root having a u/m value of 1.500 is shown in figure 1, *E*. As the u/m index approaches unity, the root is nearly spherical (fig. 1, *A*).

Another shape variation that may occur in beet roots having the same a/d index is shown in figure 1, *A*, *F*, and *G*. This results from differences in root thickness at the base of the thickened portion of the beet. An index of shape for measuring this type of variation was derived by dividing the root diameter (ld) by the equatorial diameter of the thickened portion of the beet (d). A small value for the ld/d index indicates a beet with a small taproot. Such a beet appears more nearly spherical than one having the same a/d and u/m indices

EXPLANATORY LEGEND FOR FIGURE 1

Shape indices of beet roots. Factors: Equatorial diameter (d), measured by line *bc;* thickness (ld) at base of thickened portion; polar diameter (a), measured by line u+line m; thickness (ls) at top of thickened portion. *A*, Outline of beet root, with lines and letters indicating measurements taken for computation of shape indices. The root illustrated has the following shape indices: a/d, 1.000; u/m, 1.000; and ld/d, 0.200. *B*, Outline of a flattened root having the lowest a/d value for an individual root in the material studied. The drawing represents a root having the following indices: a/d, 0.650; u/m, 1.000; and ld/d, 0.200. *C*, Outline of the longest individual beet in the material studied, having the following indices: a/d, 1.200; u/m, 1.000; and ld/d, 0.200. *D*, Outline of the most top-shaped individual root in the material studied, having the following indices: a/d, 1.000; u/m, 0.400; and ld/d, 0.200. *E*, Outline of most inverted top-shaped individual root in the material studied, having the following indices: a/d, 1.000; u/m, 1.500; and ld/d, 0.200. *F*, Outline representing a root having an ld/d index equal to the smallest value for this index in the roots studied. Indices: a/d, 1.000; u/m, 1.000; and ld/d, 0.100. *G*, Outline representing a root having an ld/d index equal to the largest value for this index in the roots studied. Indices: a/d, 1.000; u/m, 1.000; and ld/d, 0.300. *H*, Outline representing the most flattened root that could result from the extremes of indices for individual roots. The drawing is a composite of the extremes in indices that would contribute to a flat root. Indices: a/d, 0.650; u/m, 1.000; and ld/d, 0.100. *I*, Outline representing the longest root that could result from the extremes of indices for individual roots. The drawing is a composite of the extremes in indices that would contribute to a long root. Indices: a/d, 1.200; u/m, 1.000; and ld/d, 0.300.

but with a large, thick taproot. A beet having a small *ld/d* index (0.100) is shown in figure 1, *F*, and one with a large *ld/d* index (0.300) is shown in figure 1, *G*. A beet having a taproot of medium thickness, with an *ld/d* index of 0.200, is shown in figure 1, *A*; *A*, *F*, and *G* differ only in the *ld/d* index.

METHOD OF ANALYSIS

These studies were originally planned so that the data could be analyzed statistically by Fisher's analysis of variance method (*4*). However, drought, excessive rain, poor stands, and other factors made it impossible to record the data for a complete design as planned. Consequently, the results were not well adapted for analysis by the variance method. A study of the data indicated that in most cases comparisons could be made more satisfactorily by Student's method (*8*).

EFFECT OF VARIOUS FACTORS ON SHAPE

SIZE

For the study of the influence of size on the shape indices, twelve 100-root samples of large beets and twelve 100-root samples of small beets were harvested from 11 populations, as follows: One sample of large and one of small beets from each of the two plantings at Arlington Experiment Farm in 1933; from both light and heavy soil from the two plantings at Beltsville, Md., in 1933; from each of two plantings at Arlington Experiment Farm in 1934; and from plots on both heavy and light soil from the first planting at Beltsville in 1934; and two samples of large and two of small beets from the crop grown at Beltsville in 1935. In each case the large- and small-beet samples to be compared were harvested from the same population and had been grown as nearly as possible under the same environmental conditions.

The means for the two size samples from the same population were paired for statistical analysis by Student's (*8*) method. The mean diameters of the two size samples varied with the different populations; however, in each population the beets in the large-size sample had a mean diameter approximately 2 cm. greater than the mean diameter of the beets in the corresponding small-size sample. Reference to table 1 will show that the 12 pairs of samples used in this study of the influence of the size on shape of root were taken from populations grown under a wide range in soil texture, moisture supply, and season of planting and harvesting.

A summary of the data on the influence of size on the three shape indices, analyzed by Student's (*8*) method, is presented in table 2. The odds are 9,999 to 1 that beets of small equatorial diameter (*d*) have greater *a/d* and *ld/d* indices than those having a large diameter. The odds are 19 to 1, or barely significant, that roots of large equatorial diameter have greater *u/m* indices than roots having a small equatorial diameter.

These data show that the size of the root as measured by its equatorial diameter (*d*) must be taken into consideration in the study of variation in shape of beet roots.

TABLE 2.—*Influence of size on shape of beet roots* [1]

Index	Mean index value		Difference	Odds
	Large beets	Small beets		
a/d	0.905	0.936	0.031	[2] 9,999:1
u/m	.658	.631	.027	[3] 19:1
ld/d	.185	.215	.030	[2] 9,999:1

[1] 12 samples of large beets and 12 of small beets were compared in each test.
[2] Odds that small beets have greater indices than large ones.
[3] Odds that large beets have greater indices than small ones.

SOIL TEXTURE

The 100-root samples taken for the study of the influence of soil texture on shape were all graded to size. All the populations were grown under conditions as nearly the same as possible, except that one lot of samples was taken from plots on heavy soil and the other lot of samples from plots on light, sandy soil. The samples had a mean diameter of approximately 6 cm. Ten pairs of means from 100-root samples were studied by Student's (*8*) method. A summary of the data is given in table 3.

TABLE 3.—*Influence of soil texture on shape of beet roots* [1]

Index	Mean index value		Difference	Odds [2]
	Heavy soil	Light soil		
a/d	0.908	0.941	0.027	61:1
u/m	.674	.632	.042	100:1
ld/d	.234	.198	.036	1,110:1

[1] 10 samples from heavy soil and 10 from light soil were compared in each test.
[2] Odds that heavy soil produces beets of greater *a/d*, *u/m*, and *ld/d* indices.

The odds are 61 to 1 that beets when grown on heavy soil have greater *a/d* indices than when grown on light soil; 100 to 1 that heavy soil produces beets having greater *u/m* indices than beets of the same size grown on light soil; and 1,110 to 1 that the *ld/d* index is greater for beets grown on heavy soil than for those grown on light soil.

FERTILIZER

All of the material for the fertilizer studies was grown at Beltsville in 1933 and 1934. The 10 samples from each fertilizer treatment consisted of 1 sample from heavy and 1 from light soil from the 1933 crop, and 2 samples from the 2 soil types from both of the crops grown in 1934.

Representative samples were harvested from plots in each of the three fertilizer treatments in each of the crops studied. Ten 100-root samples from each of the three fertilizer treatments were used in pairing for statistical analysis.

In analyzing the data by Student's (*8*) method, the mean index for the 100-root samples from one fertilizer treatment was paired with that for a similar sample from the same crop but from a different fertilizer treatment. For each crop the mean index for 100 roots

from treatment 1 was paired with that for 100 roots from treatment 2; the mean index for treatment 2 was then paired with that for treatment 3. Finally, the mean indices for samples from treatments 1 and 3 were paired, thus giving 3 sets of pairs for each of the 10 samples from each fertilizer treatment.

A summary of the data from the fertilizer studies is given in table 4. In no case was the difference due to the fertilizer treatment significant.

TABLE 4.—*Influence of fertilizer treatment on shape of beet roots* [1]

Index	Mean index value for treatment—			Treatments paired	Difference	Odds
	1	2	3			
e/d	0.934	0.927	0.914	1 and 2	0.007	3:1
				2 and 3	.013	11:1
				1 and 3	.020	18:1
u/m	.665	.657	.681	1 and 2	.008	1:1
				2 and 3	.023	9:1
				1 and 3	.016	5:1
ld/d	.205	.209	.216	1 and 2	.004	2:1
				2 and 3	.007	3:1
				1 and 3	.011	4:1

[1] 10 samples from each of 2 fertilizer treatments were compared in each test.

Less time was required for the beets on the heavily fertilized plots to reach a marketable size than on the less fertile plots. No doubt significant differences in shape indices could be shown between random samples harvested on the same date from the three treatment plots, resulting from the differences in growth rate caused by the treatments. However, the data in table 4 show that the indices for beets of the same size were little affected by the treatments.

SEASON

For the study of the influence of time of planting on shape, 100 roots of the same diameter were harvested from each of the four crops grown at the Arlington Experiment Farm in 1934 and from each of the four crops grown at Beltsville in 1935 and 1937. In 1934 and 1935 the 100-root samples were obtained by combining 4 samples of 25 roots each harvested from the same plots but from different parts of each plot. In 1937 a sample of 100 roots taken from the entire population was used. The 100-root samples were considered to be representative of the respective populations. It is obvious that great variation in climatic conditions may occur during the same season in different years. Data on the effect of season on root shape are available for only 3 years. Additional data would be needed to enable one to draw reliable conclusions as to seasonal influence on the shape of roots. While the seasonal data are too limited to justify general conclusions, they indicate that beet roots grown late in the season when the day length is becoming shorter and temperatures are declining have a greater length as determined by the *a/d* index than roots grown earlier in the season when temperatures are high and the day is longer.

The thickness of the taproot, as determined by the *ld/d* index, appears to be greatest in beets grown late in the season.

A summary of the data is given in table 5.

TABLE 5.—*Influence of date of planting on shape of beet roots*

Date of planting	Date harvested	Diameter	Mean shape index [1]		
			a/d	*u/m*	*ld/d*
		Cm.			
Apr. 9, 1934	July 3, 1934	5.52±0.007	0.896±0.009	0.594±0.009	0.181±0.004
May 9, 1934	Aug. 24, 1934	5.54± .038	.930± .010	.585± .007	.143± .005
June 7, 1934	Sept. 14, 1934	5.52± .056	.921± .012	.675± .010	.153± .004
Aug. 1, 1934	Oct. 12, 1934	5.53± .111	.954± .015	.758± .007	.160± .003
May 8, 1935	July 2, 1935	5.08± .027	.923± .015	.590± .005	.181± .009
June 4, 1935	Aug. 6, 1935	4.97± .015	.962± .011	.637± .011	.177± .005
July 9, 1935	Sept. 15, 1935	5.03± .031	.955± .008	.761± .007	.213± .007
Aug. 5, 1935	Oct. 11, 1935	4.98± .047	.994± .012	.743± .009	.272± .004
June 19, 1937	July 23, 1937	4.97± .027	.978± .010	.585± .009	.183± .010
July 19, 1937	Sept. 28, 1937	4.98± .030	.990± .013	.734± .007	.195± .005
July 30, 1937	Sept. 30, 1937	4.96± .028	1.082± .011	.719± .008	.245± .004
Sept. 23, 1937	Nov. 5, 1937	4.93± .045	1.113± .008	.629± .010	.300± .006

[1] Values represent mean index and probable error.

In 1934 the differences in the mean *a/d* indices for the four crops were not significant except between the first and fourth crops; in this case the difference was 3.3 times its probable error. The same condition was noted in 1935. The difference between the mean *a/d* indices for the first and fourth crops was the only difference too great to be attributable to chance alone. In 1937 there was a significant increase in the *a/d* index for each successive crop except the second. In all 3 years the first of the four crops had the smallest *a/d* index and the last crop the largest; the intermediate crops did not show a consistent trend, except in 1937.

Of the three indices the *u/m* were the least consistent. The first crop in 2 of the 3 years had the smallest *u/m* index; but the last crop did not have the largest. There appears to be a tendency for beets grown during the early part of the season to be more top-shaped (fig. 1, *D*) than roots grown later in the season.

Beets grown late in the season tend to have thicker taproots as indicated by the *ld/d* index than those grown earlier in the season. Except for the large *ld/d* index for the first crop in 1934, the trend from a small to a larger value was fairly consistent. With the exception of the first crop in 1934, the *ld/d* index for the last crop was much larger than for the first. The large *ld/d* index for the first crop in 1934 may be due to the fact that the planting date was a month earlier than the first crop in 1935, and over 2 months earlier than in 1937. The low temperature and short day when the first 1934 crop was developing may have contributed to the large taproots in this case. However, the trend of the *a/d* and *u/m* indices of this crop was not affected by early planting.

Although the data recorded show a trend toward larger values for all of the three indices as the date of planting is delayed, it should be emphasized again that, in view of the great differences in environmental conditions that may occur at the same time of year in different seasons, definite conclusions as to the influence of season on the shape indices cannot be drawn from only 3 years' data.

DISCUSSION

The analysis of the recorded data shows that there are significant differences in the shape of beet roots that can be attributed to environmental factors. Although the differences in mean indices for 100-root

samples were in many cases shown to be statistically significant, the differences in shape were often so small that it was impossible to recognize them by visual inspection of the entire samples. The extremes in magnitude of the individual root indices found in the large number of individual roots studied are shown in figure 1, *B*, *C*, *D*, *E*, *F*, and *G*. All of the drawings shown in figures 1 and 2 represent beet roots having the same equatorial diameter (*d*). The values 1.000, 1.000 and 0.200 for *a*/*d*, *u*/*m*, and *ld*/*d* indices, respectively (fig. 1, *A*), were used as the basic values for all of the drawings except for figure 1,

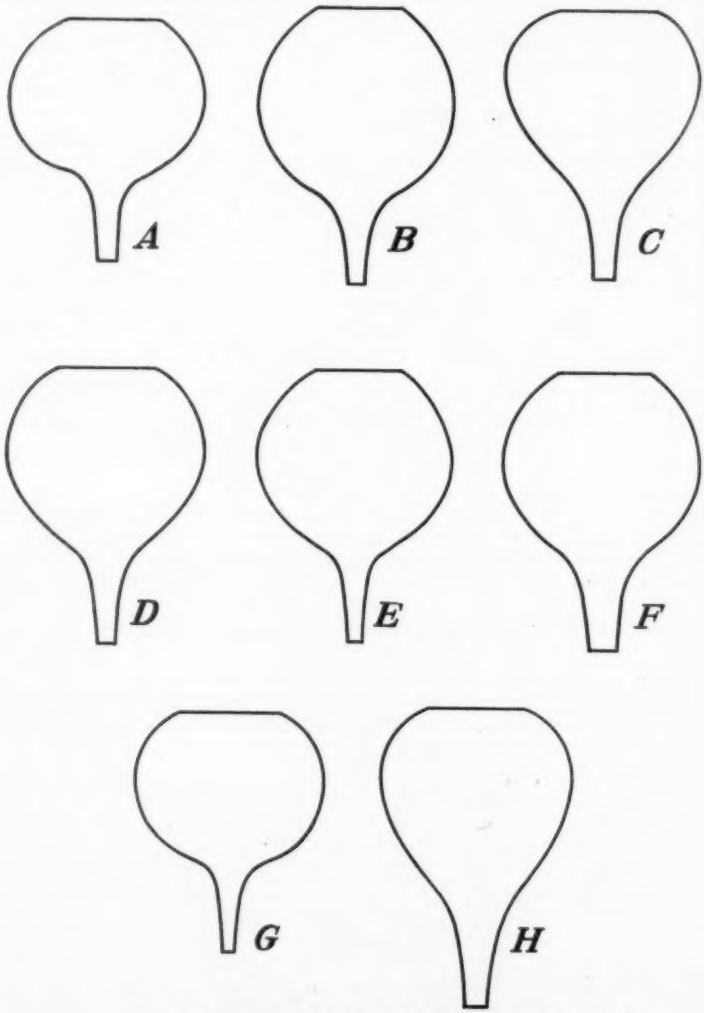

FIGURE 2.—FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

H and *I*, and figure 2, *G* and *H*. These values were kept constant except for one index in each drawing. Figure 1, *B* and *C*, indicates the extremes in *a/d* values for single roots in the many roots studied. The smallest *a/d* value for an individual root was 0.650 (*B*) and the largest 1.200 (*C*). Figure 1, *D* and *E*, shows the extremes in the *u/m* index for individual roots. The least *u/m* value for a single root was 0.400 (*D*) and the largest, 1.500 (*E*). The extremes in the *ld/d* index were 0.100 (*F*) and 0.300 (*G*).

The outline drawings *A* to *F* in figure 2 show the extremes in the mean indices for the 100-root samples. The smallest mean *a/d* index for the 100-root samples was 0.840 for a sample from the first crop at Arlington in 1933 (fig. 2, *A*), and the largest was 1.080 for 100 roots from the second crop on heavy soil at Beltsville in 1934 (*B*). The extremes in the *u/m* index for 100-root samples were 0.520 for a sample from the first crop at Arlington in 1933 (*C*) and 0.726 for a sample from the first crop on heavy soil at Beltsville in 1934 (*D*). The extremes in the *ld/d* index for the 100-root samples were 0.147 for the second crop on light soil at Beltsville in 1934 (*E*) and 0.279 for a sample from the crop on heavy soil at Beltsville in 1933 (*F*).

The outline drawings in figure 2, *G* and *H*, are composites showing the greatest variation in shape that could result from the extremes in mean indices for the 100-root samples. *G* represents the most nearly spherical beet that could result from the extremes in means for the three indices within the material studied. *H* indicates the longest root that could result from the extremes in mean indices for 100 roots.

It may be assumed that *G* and *H* in figure 2 represent somewhere near the extremes in mean shape for the 100-root samples that might be expected to result from this particular strain under the range of environmental conditions that prevailed during the 5 years of study. If every beet in a sample approached the shape of *G* in figure 2 and those in another sample approached the shape of *H*, it would not be difficult to recognize a difference in the mean shape of the two samples by visual inspection. With *G* and *H* representing the extremes in means for samples, it is obvious that the variation in all three indices that may occur in individual roots within a sample makes it prac-

EXPLANATORY LEGEND FOR FIGURE 2

Shape indices of beet roots. *A*, Outline representing a beet root having an *a/d* index equal to the smallest mean *a/d* index for a 100-root sample among the samples studied. Indices: *a/d*, 0.840; *u/m*, 1.000; and *ld/d*, 0.200. *B*, Outline representing a beet root having an *a/d* index equal to the largest mean *a/d* index for a 100-root sample. Indices: *a/d*, 1.080; *u/m*, 1.000; and *ld/d*, 0.200. *C*, Outline representing a beet root having a *u/m* index equal to the smallest mean *u/m* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 0.520; and *ld/d*, 0.200. *D*, Outline representing a beet root having a *u/m* index equal to the largest mean *u/m* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 0.726; and *ld/d*, 0.200. *E*, Outline representing a beet root having an *ld/d* index equal to the smallest mean *ld/d* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 1.000; and *ld/d*, 0.147. *F*, Outline representing a beet root having an *ld/d* index equal to the largest mean *ld/d* index for a 100-root sample. Indices: *a/d*, 1.000; *u/m*, 1.000, and *ld/d*, 0.279. *G*, Outline representing a beet root having indices equal to the three mean indices for 100-root samples that were most indicative of spherical-shaped roots. Indices: *a/d*, 0.840; *u/m*, 0.726; and *ld/d*, 0.147. *H*, Outline representing a beet root having indices equal to the three mean indices for 100-root samples that were most indicative of long-shaped roots. Indices: *a/d*, 1.080; *u/m*, 0.520; and *ld/d*, 0 279.

tically impossible in many cases to arrive at any estimate of the mean shape of a sample of root except by very careful measurement and the computation of indices. The comparison of samples by visual inspection is much more difficult if the samples consist of roots of various sizes, since it has been shown that the size of root is one of the most consistent factors influencing the index values.

The results of these studies indicate that significant differences in shape of beet roots may result from environmental causes. However, in a uniform strain of beets, such as that of Detroit Dark Red used in this work, the differences in shape indices for representative root samples may be very small except in cases where the crop is grown under wide extremes of soil, climatic, or seasonal conditions.

SUMMARY AND CONCLUSIONS

These studies included the influence of size of root, soil texture, soil fertility, and season of planting on the shape of beet roots.

Three indices of shape were computed for individual roots in samples of 100 roots each, harvested from 17 crops of the variety Detroit Dark Red, and grown under a wide range of environmental conditions over a period of 5 years.

All three of the indices were found to be influenced by the size of the roots. Small beets were longer and more top-shaped and had thicker taproots, in proportion to their equatorial diameter, than large beets.

Beets grown in heavy clay soil were longer and had thicker taproots, n relation to their equatorial diameter, than roots grown in deep sandy soil.

Wide extremes in soil fertility, as judged by the rates of fertilizer application, failed to produce significant differences in any of the three shape indices for roots of the same size as determined by the equatorial diameter.

Although the data on the influence of season of planting are limited, they indicate that the roots from crops planted in late summer tended to be longer, and thicker at the plane *ld*, than those from crops grown in the spring and early summer.

While the differences in the shape indices for samples of 100 roots grown under different environmental conditions were found to be statistically significant, the differences between means were in many cases very small.

Variation in the shape of individual roots within a sample was too great to permit a satisfactory estimate by visual inspection of the shape of a representative sample from a population.

It is concluded from these studies that the shape of roots of a good strain of beets of the variety Detroit Dark Red may be altered by environmental conditions and that these variations can be shown by numerical shape indices. However, variations in shape are not great except when the crops are grown under wide extremes of soil, climatic, and seasonal conditions.

Difference in size of the individual roots composing the sample seems to be the most consistent cause of the differences in shape that appear to exist between random samples from populations grown under different environmental conditions. No satisfactory study of environmental influences on the shape of beet roots can be made on

random samples unless the size of the individual root is taken into account.

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RELATIVE INFLUENCE OF THE VARIOUS FACTORS THAT AFFECT EGG PRODUCTION OF TURKEYS DURING DIFFERENT PARTS OF THE FIRST LAYING YEAR [1]

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INTRODUCTION

In a previous paper (*1*) [3] the writer showed the influence of various factors on first-year egg production in turkeys. These factors, in the order of their importance, were date of last egg (persistency), pauses (total length of all 7-day broody and nonbroody pauses), date of first egg (sexual maturity), and rate measured by percent production, exclusive of pauses, during March and April.

Egg production during the season when hatching eggs are wanted is of much greater practical importance than later in the year. The demand for hatching eggs does not come during a fixed period, however, since it varies from year to year and also to some extent in different geographic areas. In California most of the eggs are set before the end of April, but in some years eggs are set in May and even June. The price paid for hatching eggs usually decreases as the hatching season advances. The greater value of early eggs as compared with those laid late in the season suggested the need for information regarding the factors that influence egg production in different periods of the first laying year. A statistical analysis of turkey egg records was accordingly undertaken to determine the relative influence of the various factors previously correlated with total first-year egg production on egg production during different parts of the first laying year. This paper presents the results of that analysis.

MATERIALS AND METHODS

The data are for the same birds used by the writer (*1*) to determine the influence of different factors on total first-year egg production. The various measures of the factors correlated with egg production have already been described. It is sufficient here to recall that (1) date was in each case coded and stated as the number of days from the first of the year (in the case of date of hatch this was the number of days from the first of the year in which the bird was hatched; in the case of date of first egg and date of last egg it was the number of days from the first of the year in which the record was made, and this was in each case the year following the one in which the bird was hatched); (2) pauses were all periods of nonproduction of 7 or more days regardless of whether or not the bird became broody; (3) net rate was the percent production while laying, pauses of 7 or more days being excluded when calculating net rate. Net rate for May

[1] Received for publication October 31, 1938.

[2] The writer is indebted to Ward S. Watson, who performed most of the computations, and to the Works Progress Administration, under whose auspices part of the tables were checked.

[3] Italic numbers in parentheses refer to Literature Cited, p. 753.

and June and after the end of June was in each case calculated for birds that laid six or more eggs in the given period, which reduced the sample to less than the total number of records used for other calculations.

The first laying year was divided arbitrarily into three periods (A) to the end of April, (B) May and June, (C) after the end of June (table 1). The means and standard deviations for first-year egg production, for the date of hatch, and for the various factors based on the first laying year have been presented elsewhere (*1*), together with the net spring rate for March and April. The means and standard deviations for egg production, number of pauses, length of pauses, and net rate in each of the periods considered in this paper are presented in table 1. Net rate for March and April was used for the period to the end of April. It will be observed that egg production decreased in each successive period, but since the periods were not of equal length no particular significance can be attached to this finding. The net rate also decreased. The number of pauses per bird showed a tendency to increase slightly after the end of June, but the length of pauses per bird varied irregularly.

Zero order coefficients of correlation were calculated from grouped data. Sheppard's correction was not applied. Coefficients of multiple correlation were calculated by the method of Wallace and Snedecor (*8*). Formulas for all other calculations used are described by Ezekiel (*3*).

TABLE 1.—*Mean and standard deviation for egg production per bird, for number and length of pauses per bird, and for net rate, during different parts of the first laying year; 1934–36*

Period and year [1]	Egg production		Pauses		Length of pauses		Net rate	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
A, to the end of April:	*Number*	*Number*	*Number*	*Number*	*Days*	*Days*	*Percent*	*Percent*
1934	36.1	13.4	0.7	0.7	13.9	19.2	[2] 71.1	5.8
1935	40.2	15.3	.5	.7	14.0	11.8	[2] 68.8	9.6
1936	38.7	13.9	.7	.7	20.2	23.3	[2] 99.1	9.6
B, in May and June:								
1934	24.1	13.0	.6	.8	12.6	15.7	59.6	9.0
1935	23.4	11.5	1.1	.9	22.0	14.3	61.8	10.1
1936	25.2	13.3	.6	.7	10.7	12.6	63.5	9.8
C, after the end of June:								
1934	18.0	15.2	1.1	1.2	16.5	16.8	52.3	9.7
1935	17.1	9.9	.8	1.1	14.5	17.4	53.5	14.4
1936	15.6	12.3	.8	1.1	11.7	14.8	57.9	9.5

[1] The total number of birds for which records were available was as follows: 1934, 91; 1935, 74; 1936, 73.
[2] Spring net rate for March and April only.

STATISTICAL ANALYSIS

The zero order coefficients of correlation for egg production during the first laying year and during parts of the first year were computed, and thev alues of $\bar{r}$, the coefficient most probably representing the true value for the universe from which the sample was drawn, are given in table 2. There was a high correlation between total egg production and egg production during any part of the year. This correlation is, of course, partly spurious because the whole is correlated with a part. There is, however, a highly significant correlation between egg production to the end of April and the egg production

in May and June. The correlation between egg production before the end of April and after the end of June is low and of doubtful significance. Egg production in May and June is apparently correlated with egg production after the end of June, but the coefficients vary from a low value in 1934 to highly significant values in 1935 and 1936. The low and variable egg production after the end of June indicates that egg production in this period is adversely affected by hot weather. This is reflected in reduced egg production and increased variability of egg production after the end of June (table 1).

TABLE 2.—*Zero order coefficients of correlation—number of eggs laid during the first year and during different parts of the first laying year—values of* $\bar{r}$, *1934–36*

Egg-production periods correlated	1934	1935	1936
Year; to end of April	0.751	0.813	0.692
Year; May and June	.751	.806	.813
Year; after the end of June	.650	.692	.627
To end of April; May and June	.474	.508	.399
To end of April; after the end of June	.170	.275	.000
May and June; after the end of June	.163	.455	.338

TABLE 3.—*Zero order coefficients of correlation—values of* $\bar{r}$*—for various factors correlated with egg production in different parts of the first laying year, 1934–36*

Variables correlated with egg production in periods A, B, and C	A, egg production to the end of April			B, egg production during May and June			C, egg production after June		
	1934	1935	1936	1934	1935	1936	1934	1935	1936
Date of hatch	−0.228	−0.259	0.000	−0.157	−0.128	0.170	0.000	−0.269	0.106
Age at first egg	−.403	−.425	−.567	.000	.000	−.093	.000	.000	.000
Date of first egg	−.791	−.757	−.655	−.109	.228	.000	.000	.000	.000
Age at last egg	.320	.380	.000	.383	.478	.296	.727	.705	.805
Date of last egg	.262	.324	.000	.347	.504	.355	.768	.728	.859
Length of laying year	.443	.578	.261	.326	.458	.375	.746	.615	.812
Rate (year)	.000	.144	−.060	−.090	.327	.237	.000	.000	.000
Spring rate (March and April)	.170	.268	.140	.059	.385	.292	.194	.284	.213
Rate in May and June	.000	.000	−.032	.097	.526	.289	.257	.381	.000
Rate after June	−.209	−.108	−.119	−.294	.000	.000	.054	−.105	−.142
Pauses:									
Number (year)	.000	−.044	−.171	.000	−.088	−.132	.210	.000	.206
Length (year)	−.198	−.342	−.508	−.449	−.296	−.526	.178	.000	.186
Number to end of April	−.226	−.504	−.350	−.365	−.441	−.246	−.082	−.142	.000
Length to end of April	−.459	−.557	−.557	−.467	−.475	−.506	.044	−.152	.053
Number in May and June	.000	.000	.000	−.090	−.136	−.187	.000	.000	−.039
Length in May and June	.000	−.135	−.062	−.370	−.163	−.310	.139	.000	−.042
Number after June	.176	.152	.000	.112	.191	.000	.323	.210	.372
Length after June	.000	.166	.000	.000	.139	.000	.051	.000	.300

Table 3 gives the values of $\bar{r}$ for the various factors correlated with egg production in different parts of the first laying year. Egg production to the end of April was correlated with date of and age at first egg, the coefficient of correlation being higher for date of first egg than for age at first egg. The coefficients of correlation for age and date of last egg with egg production to the end of April were low in 1934 and 1935, and nil in 1936. Length of laying year was also correlated with egg production in this period, but the coefficient is less than for date of first egg. Length, but not number, of pauses during the year is correlated with egg production to the end of April, whereas rate during the year is not. Number of pauses and length of pauses to the end of April and also net spring (March and April) rate are all

correlated with egg production in this period and the coefficients are all higher than the corresponding correlations calculated from data for the year as a whole. These three factors for the two later periods (May and June, after June) are not correlated with egg production to the end of April. To summarize, egg production to the end of April is correlated with sexual maturity (particularly as measured by date of first egg), with number and length of pauses to the end of April, and with length of pauses during the year, with net spring rate, and, except in 1936, with persistency (date or age at last egg).

Egg production in May and June is correlated with persistency (date of or age at last egg), and with length of pauses for the year and for the first two periods but not the last period (after June). Egg production in this period is also correlated with net spring rate and with net rate in May and June in the years 1935 and 1936, but in neither case is the coefficient for the 1934 data significant. These results, while not conclusive, indicate that net rate prior to the end of June usually has some influence on egg production in May and June. Date of or age at first egg (sexual maturity) and number of pauses are not correlated with egg production in this period (table 3).

Egg production after the end of June (table 3) is correlated with age at and date of last egg and with net spring (March and April) rate, and, of course, also with length of laying year. The correlation with other factors is nil or of doubtful significance. The high correlation with length of laying year is to be expected since length of laying year is determined by date of first and last egg (or age at first and last egg). The positive correlation with number of pauses after the end of June is probably partly due to the inclusion of birds that did not lay in this period and their classification as nonpausers.

The coefficients of multiple correlation R and $\overline{R}$, the most probable values for the universe from which the sample was drawn, were determined for egg production in each of the three periods and the four factors previously used in a study of first-year egg production (*1*), namely, date of first egg, length of pauses during the year, net spring (March and April) rate, and date of last egg. It is evident from the values of $\overline{R}$ presented in table 4 that these factors accounted for nearly all the variance in egg production to the end of April but that they did not fully account for variance in May and June, nor after June. Date of first egg has the greatest influence on egg production to the end of April; the other factors appear to have a lesser influence, but, nevertheless, in each case increase the coefficient of multiple correlation slightly. Egg production after the end of June is apparently determined mostly by date of last egg (persistency).

In order to find more precisely what the relative importance of these four factors was in determining egg production in the three periods under consideration, the coefficients of partial determination were calculated. These are presented in table 5. This table shows the influence on egg production in each of the three periods of the same four factors used in table 4—date of first egg, length of pauses during the year, net spring (March and April) rate, date of last egg.

The data in table 5 show that date of first egg has the greatest influence on egg production to the end of April. The order of importance for the other factors is length of pauses, date of last egg, and spring rate.

TABLE 4.—*Coefficients of multiple correlation—values of R—for various factors correlated with egg production in different parts of the first laying year; 1934–36*

Variables correlated with egg production in periods A, B, and C	A, egg production to end of April			B, egg production in May and June			C, egg production after June		
	1934	1935	1936	1934	1935	1936	1934	1935	1936
Date of first egg; date of last egg	0.809	0.774	0.654	0.349	0.514	0.338	0.785	0.725	0.862
Date of first egg; length of pause	.626	.615	.786	.469	.361	.522	.164	.000	.152
Date of first egg; rate	.811	.847	.696	.121	.481	.270	.180	.301	.183
Date of last egg; length of pause	.476	.398	.564	.797	.733	.836	.787	.818	.869
Date of last egg; rate	.286	.385	.074	.338	.587	.412	.770	.745	.858
length of pause, rate	.245	.398	.328	.471	.446	.602	.248	.262	.286
Date of first egg; date of last egg; length of pause	.893	.873	.837	.800	.731	.835	.805	.824	.871
Date of first egg; date of last egg; rate	.825	.850	.691	.340	.612	.398	.787	.741	.962
Date of first egg; length of pause;	.853	.879	.820	.490	.520	.597	.238	.279	.274
Date of last egg; length of pause; rate	.495	.605	.567	.798	.750	.846	.790	.819	.868
Date of first egg; date of last egg; length of pause; rate	.909	.908	.852	.801	.744	.845	.809	.821	.870

TABLE 5.—*Influence on egg production in different parts of the first laying year of the four factors used in table 4*

[Reduction in unexplained variance]

Variables correlated with egg production in periods A, B, and C	Factors already considered	A, egg production to end of April			B, egg production in May June			C, egg production after June		
		1934	1935	1936	1934	1935	1936	1934	1935	1936
Date of first egg	Date of last egg; length of pause	0.739	0.718	0.559	0.011	0.000	0.000	0.076	0.028	0.015
Date of last egg	Date of first egg; length of pause	.365	.293	.214	.538	.464	.585	.638	.681	.753
Length of pause	Date of first egg; date of last egg	.414	.409	.476	.589	.367	.659	.083	.323	.063
Date of first egg	Date of last egg; rate	.651	.675	.475	.002	.047	.000	.064	.000	.027
Date of last egg	Date of first egg; rate	.063	.017	.000	.103	.187	.092	.607	.503	.734
Rate [1]	Date of first egg; date of last egg	.072	.309	.088	.000	.151	.050	.010	.048	.000
Date of first egg	Length of pause; rate	.710	.729	.545	.023	.089	.000	.000	.010	.000
Length of pause	Date of first egg; rate	.201	.191	.364	.229	.051	.306	.025	.000	.043
Rate [1]	Date of first egg; length of pause	.142	.320	.141	.026	.161	.115	.031	.078	.053
Date of last egg	Length of pause; rate	.197	.247	.058	.534	.454	.554	.600	.646	.732
Length of pause	Date of last egg; rate	.178	.256	.317	.591	.333	.658	.077	.260	.068
Rate [1]	Date of last egg; length of pause	.024	.246	.003	.0067	.054	.056	.014	.001	.000
Date of first egg	Date of last egg; length of pause; rate.	.769	.722	.597	.012	.000	.000	.078	.012	.015
Date of last egg	Date of first egg; length of pause; rate.	.362	.227	.165	.529	.388	.555	.633	.647	.738
Length of pause	Date of first egg; date of last egg; rate.	.456	.364	.475	.595	.285	.659	.000	.279	.057
Rate [1]	Date of first egg; date of last egg; length of pause.	.138	.257	.088	.007	.040	.051	.017	.000	.000

[1] Stands for net spring (March and April) rate.

Egg production in May and June is apparently influenced about equally by length of pause and date of last egg. Spring rate and date of first egg have relatively much less influence on egg production in these months (table 5).

Of all the factors considered, date of last egg (persistency) has by far the most important influence on egg production after June (table 5). The other factors appear to have little influence on egg production after June, although the values for length of pause tend to be higher than those for date of first egg and spring rate.

DISCUSSION

The data presented indicate that the various factors which were correlated with egg production are not of equal importance in determining egg production in different parts of the first laying year.

Thus sexual maturity (date of first egg) is the most important factor influencing egg production to the end of April, whereas its influence on egg production is slight in May and June and negligible after the end of June. On the other hand, persistency, as measured by date of last egg, has greater influence than any other factor on egg production after June, an important influence on egg production in May and June, but relatively little influence on egg production prior to the end of April. Length of pauses has an important influence on egg production in the first two periods, and the data presented indicate that there is also some influence on egg production in the last period, after the end of June. These results thus confirm the earlier findings that the time factors have an important influence on first-year egg production but they show that, except perhaps for pausing, their influence is not exerted equally throughout the year. Sexual maturity and persistency have previously been shown to be independent variables in turkeys (*1*) and in chickens (*4*). The data presented in this paper show that there is little overlapping of their influence on egg production in the different periods, with sexual maturity influencing egg production early in the year while persistency influences egg production more later in the year.

Net spring rate appears to have less influence on egg production than sexual maturity, pauses, and persistency.

Net spring rate is positively correlated with egg production in each of the three periods, although some of the coefficients are low. The coefficients of correlation for net rate in May and June and egg production in May and June are above those for net spring rate and egg production in May and June except in 1936. Nevertheless, table 3 shows that net spring rate is more consistently positively correlated with egg production in the different periods than net rate for May and June or for after June. It has previously been shown that net spring rate is more closely correlated with first-year egg production than is net rate calculated for the year (*1*). From this evidence it may be concluded that net spring (March and April) rate is a better measure of rate than net rate calculated for the entire year, for May and June, or for after June.

Both number and length of pauses in the first two periods are negatively correlated with egg production in these periods. These factors for the first period are more closely correlated with egg production in the first two periods than those based on the entire year or on the two later periods. After the end of June, egg production tends to be positively correlated with length and number of pauses. Birds that lay more than a few eggs after the end of June almost invariably pause. Since, as stated above, birds that did not lay in this period or laid only a few eggs and then stopped, were classified as nonpausers, a positive correlation is to be expected. In this connection it should be recalled that length of pauses for the year is correlated with persistency (date of last egg). No particular significance can be attached to the positive correlation between length of pauses and egg production after June. The correlation is probably the result of an unfavorable environment which causes low egg production after June. (See previous discussion and Asmundson and Lloyd (*2*).) The low egg production after June may also result from the tendency to lay for only a short period, which is characteristic of some species. (See Rowan (*6*) and others.) This can only be a minor factor, however,

since young turkey hens lay late in the summer and some of these lay in the autumn. There is good evidence to show that temperature has little influence on the initiation of egg production in turkeys (7). Once egg production has started, however, unfavorable temperatures may cause a cessation in egg production. High temperatures in July and August are probably one of the causes of low egg production after June. The apparently great influence of the unfavorable environment on egg production after June may account for the low correlation of egg production in this period with length of pauses and net rate. The relatively low correlation between egg production after June and egg production in the earlier periods may also be due to differences in the weather in the three periods.

The relatively low correlation of egg production after June with spring egg production, together with the relatively slight influence of persistency on spring egg production, would indicate that the practical turkey breeder could ignore egg production after June but should select for the three factors that influence spring egg production most. These, in the order of their importance, are: (1) Sexual maturity, which is best measured by date of first egg; (2) pauses, absence of pauses being desirable; and (3) net rate. Although net rate appears to be the least important of these, it would seem well worth while to select for improved rate in view of the variation shown to occur in this character in this paper (table 1) and by Marsden (*5*).

SUMMARY

The first laying year of turkey hens trap-nested in 1934, 1935, and 1936 was arbitrarily divided into three periods; (A) From beginning of egg production to the end of April; (B) May and June; and (C) after June. Egg production in each of these periods was correlated with various factors.

Sexual maturity (date of first egg) was found to be the most important factor influencing egg production to the end of April, the other factors in the order of their importance being length of pauses, date of last egg (persistency), and net rate.

Egg production in May and June was influenced most by length of pauses and date of last egg, whereas date of first egg and net spring rate had less influence on egg production in this period.

Persistency had by far the greatest influence on egg production after June while the coefficients for egg production with the length of pauses were low. Date of first egg and net spring rate had little or no influence on egg production in this period.

Persistency has little influence on egg production early in the year; hence the factors of greatest practical importance in selecting for improved egg production during the breeding season are, in the order of their importance, sexual maturity (date of first egg), length and number of all broody and nonbroody pauses, and net spring rate.

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INTERPRETATION OF MENDELIAN CLASS FREQUENCIES[1]

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INTRODUCTION

The application of the chi-square test to Mendelian class frequencies constitutes a familiar illustration of the general problem of testing a hypothesis by means of observed data. Since the test is almost universally employed by geneticists, it appears desirable to present a few principles that may serve as a guide to its interpretation.

The investigator should have no illusions regarding the ability of the test to prove or disprove the hypothesis under consideration. No such test exists. Neither should he harbor the mistaken idea that the test measures the probable truth of the hypothesis. Attempts to measure the probable truth of a hypothesis have always become involved in the controversial subject of inverse probability and have not been generally accepted by mathematical statisticans. The philosophy underlying the chi-square test is foreign to such a concept and avoids the difficulties to which it leads, but only at the expense of providing the investigator with a test that falls short of what is actually desired. The test merely shows whether or not the observed data are likely to be obtained under conditions of random sampling if the hypothesis under consideration is true. The investigator's conclusions in regard to the truth or falsity of the hypothesis can be nothing more than inferences based on the results given by the test and such other information relative to his particular problem as may be available.

THE CONCEPT OF RANDOM VARIATION

It is regrettable that the small amount of information which the chi-square test is capable of contributing to problems involving the testing of hypotheses has in the past been still further restricted by the failure of some investigators to comprehend the concept of random variation and its implications in experimental work. At least a part of the blame for this failure must be borne by the statistician, for the older chi-square tables are not adequate for an efficient application of the chi-square test. These tables tend to create the impression that only values of chi square larger than one would expect to arise by chance should be regarded as lying outside the normal range of variation.

The reason for this impression is a subject for speculation. One can hardly suppose that such statisticians as Pearson (*7*)[2] and Elderton (*1*) had any mistaken ideas as to what constitutes random variation, although it appears unlikely that any particular importance would

[1] Received for publication October 25, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 759.

have been attached to a small value of chi square at a time when the test was used principally to measure the goodness of fit of frequency curves. If any objection to too good a fit occurred to them, they appear to have made no mention of it. Fisher (*3*) was fully aware of the implications of unusually good agreement between observation and theory, but proposed no better criterion for determining the normal range of variation of chi square than was provided by Elderton's table. This circumstance may have been due to the lack of a suitable criterion for establishing limits of variability in the case of skew frequency distributions.

In order to establish a criterion that will show whether or not an observed value of chi square is within the normal range of variation, it is necessary to arrive at an agreement as to what constitutes the normal range of variation. The senior author has found that the shortest range of values which will include 95 percent of the observations may be regarded as the normal range of variation, and has computed the following chi-square table (table 1):[3]

TABLE 1.—*Critical values of chi square for 5-percent level of significance*

Degrees of freedom	Value		Degrees of freedom	Value		Degrees of freedom	Value	
	Lower	Upper		Lower	Upper		Lower	Upper
1	0.000	3.841	11	2.954	20.305	21	9.267	33.921
2	.009	5.991	12	3.516	21.729	22	9.958	35.227
3	.003	7.816	13	4.099	23.135	23	10.656	36.526
4	.085	9.530	14	4.700	24.525	24	11.361	37.818
5	.298	11.191	15	5.318	25.900	25	12.073	39.103
6	.607	12.802	16	5.948	27.263	26	12.791	40.383
7	.990	14.369	17	6.591	28.615	27	13.514	41.658
8	1.425	15.897	18	7.245	29.955	28	14.243	42.927
9	1.903	17.393	19	7.910	31.285	29	14.977	44.192
10	2.414	18.861	20	8.584	32.607	30	15.716	45.451

The principle on which table 1 is based is theoretically sound and lends itself well to the problem of establishing limits of variability for a large variety of statistics that are distributed according to skew frequency curves. It so happens that the ordinates of the chi-square distribution at the two extremities of this range are equal when the number of degrees of freedom is greater than 2. This property, which can be demonstrated mathematically, is the necessary and sufficient condition that a particular value of chi square regarded as lying outside the normal range of variation cannot have a probability of occurrence equal to, or greater than, the probability of occurrence of some value on the other side of the mode that would be accepted as being within the normal range of variation. For 1 and 2 degrees of freedom, the lower bound of admissible values of chi square lies at zero, which agrees with the known fact that observed values in the neighborhood of zero are expected to occur more frequently than any other. For an extremely large number of degrees of freedom, the chi-square distribution approaches a symmetrical form resembling the normal curve of errors. In such a case, the normal range of variation, as defined in this paper, would exclude approximately 2.5 percent of the values at

[3] Acknowledgment is made for the assistance of Kate W. Robey, of the Bureau of Animal Industry, in the construction of this table.

each extremity of the distribution, a phenomenon that has a well-known parallel in the usual treatment of the normal curve. However, this condition is not realized until the number of degrees of freedom becomes very large. For 30 degrees of freedom, the criterion presented in this paper excludes only 1.5 percent of the values of chi square at the lower extremity of the curve and 3.5 percent of the values at the upper extremity.

It is evident that the table leads to results that are perfectly consistent with the nature of the frequency distributions involved for various numbers of degrees of freedom. A similar method of approach has recently been used by Fertig and Proehl (*2*) in a consideration of Neyman and Pearson's likelihood function. In fact, the table that Fertig and Proehl (*2*) have presented for testing an observed standard deviation may be used to test an observed value of chi square in accordance with the theory embodied in Hendricks and Robey's table. For an observed value of chi square, one may compute Fertig and Proehl's k by means of the formula

$$k=0.434294\,\frac{\chi^2}{n-2}-\log\frac{\chi^2}{10n-2},$$

in which n is the number of degrees of freedom involved, and determine the probability associated with that value of k by deducting 2 units from the correct number of degrees of freedom before entering the table.

Table 1 and the table presented by Fertig and Proehl (*2*) provide more satisfactory criteria than have previously been available for deciding whether or not an observed value of chi square, as stated by Pearson (*7*), "is such that it can be reasonably supposed to have arisen from random sampling."

An investigator obtaining a value outside the normal range of variation should reconsider his hypothesis and subject his data to further scrutiny. The conclusions to be drawn in the case of a value larger than one would expect to arise by chance, are familiar to most geneticists, but the interpretation to be placed on a value smaller than one would expect may not be so apparent. Such a value does not necessarily imply either that the hypothesis is wrong or that there is something wrong with the experimental technique, for unusual sets of data occasionally arise by chance. However, any investigator who consistently obtains values of chi square smaller than would be expected to arise by chance should infer, in the case of Mendelian class frequencies, that he is working with data that were consciously or unconsciously selected to agree with the hypothesis under consideration.

DETECTION OF SELECTED DATA

In some cases, unusually close agreement between observation and theory occurs because of a natural tendency on the part of the investigator to stop his experiment when the data seem to agree with his hypothesis. The fact that such a procedure results in selected data may not have occurred to some geneticists. In this connection, it may be pointed out that, according to the law of chance, an investigator may obtain data that will satisfy any one of several different hypotheses regarding the proportion of observations to be expected

in each of the various classes. If he stops his experiment when the results seem to agree with his chosen hypothesis, his experiment cannot be regarded as a fair test of the hypothesis.

Data derived from such an experiment generally yield unusually small values of chi square because of the tendency of investigators to underestimate the amount of variability that can be accepted as arising by chance. This fact is illustrated in some data reported by Quinn and Godfrey (*8*). These data, considered en masse, consist of 44 classes of observations in which the method of selection just referred to is known to have been used. The 44 classes yield 22 degrees of freedom for which chi square has the value 6.398. By referring to table 1 it will be observed that the normal range of variation of chi square for 22 degrees of freedom is 9.958 to 35.227. The small value of chi square obtained from the data, therefore, reflects the artificial agreement between observation and theory that was imposed by the selection of the data. However, in this case, supplementary evidence is available to confirm the conclusions reported and there is no question of their validity.

The older literature in the field of genetics contains reports of data that seem to have been obtained under similar conditions. For example, Lippincott (*6*) reported three sets of data obtained from backcross matings in a study of plumage-color inheritance in chickens. From the use of only the totals in his tables, the three sets of data yield 7 degrees of freedom for which chi square has the value 0.550. The normal range of variation of chi square for 7 degrees of freedom is 0.990 to 14.369. In this illustration, there is also no question of the validity of the conclusions reported. However, such selected data are undesirable and may at times retard the discovery of genetic relationships.

A critique of Mendel's classic work recently reported by Fisher (*4*) shows how selected data may appear to substantiate an incorrect conclusion. Fisher (*4*) found unusually close agreement between observation and theory in Mendel's data. For 84 degrees of freedom, chi square had the value 41.6. In order to determine the normal range of variation, one may make use of the approximation to the chi-square distribution for large numbers of degrees of freedom suggested by Fisher (*3*). The range is 60 to 111. Therefore, the observed value, 41.6, is smaller than one would expect under conditions of random sampling and indicates that the data are a selected set. In one phase of his work, Mendel's data agree closely with the 2 : 1 ratio which he expected to obtain, but differ significantly from the correct ratio.

One other example may be cited because it illustrates another method of detecting selected data. Smith (*9*) reported data on the inheritance of annual versus biennial growth habit in sweetclover plants and concluded that the 2 factors segregated in a 3:1 ratio in the F_2 generation. The totals of his 2 classes of observations, obtained from 91 families, yield 1 degree of freedom for which chi square has the value 2.592. This result is within the normal range of variation, 0.000 to 3.841, and appears to substantiate his hypothesis. However, when the chi square test is applied to the totals of 9 subgroups of his observations, 8 containing the data for 10 families each and 1 containing the data for the remaining 11 families, chi square has the value 42.345. The normal range of variation of chi square for 9

degrees of freedom is 1.903 to 17.393. This result indicates that the data are more variable than would be expected if the hypothesis were correct and that the agreement of the totals with the hypothesis, as reported by Smith (*9*), is spurious. The same conclusion in regard to this set of data has been reported by Kirk and Immer (*5*), who made a study of the results reported for individual families.

REPORTING DATA ON POULTRY GENETICS

Poultry geneticists, as a group, apparently do not report data that agree too closely with the hypotheses tested. Warren[4] has summarized a large amount of data obtained by various investigators studying autosomal factors in the fowl.[5] Omitting data for factors involving linkage, the summary yields 213 degrees of freedom for which chi square has the value 284.4. The normal range of variation is 174 to 255. The observed value of chi square is thus larger than one would expect, but a careful examination of the data indicates that this is due to the presence of a few highly discordant observations rather than to any systematic bias in the data.

An investigator need not hesitate to report data in which the agreement between observation and hypothesis differs significantly from what is expected under conditions of random sampling if he is convinced that the discrepancy is due to chance. To avoid criticism, an investigator may be tempted to report only such results as appear to be favorable to his hypotheses and to withhold results that exhibit either less or more variability than is expected. Such a procedure, if practiced consistently, will inevitably lead to an accumulation of fictitious data that accomplishes little more than to reflect the personal opinions of the investigators who obtained it.

SUMMARY

The principles involved in the interpretation of Mendelian class frequencies are discussed. The application of the chi-square test to such data is considered as a means of detecting data which do not agree with what is expected under conditions of random sampling. A new chi-square table, which appears to be more useful for this purpose than the tables in use at the present time, is presented.

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EXPERIMENTAL TRICHOSTRONGYLOSIS IN SHEEP AND GOATS[1]

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INTRODUCTION

In 1918 Waterman (*14*)[2] described a disease occurring in goats on the island of Curaçao in the Dutch West Indies, the disease being characterized by weakness, emaciation, and profuse diarrhea. Waterman found that this disease often resulted in the death of the animal, and he considered the symptoms to be due to malnutrition combined with an infestation of small trichostrongyles, identified as *Trichostrongylus instabilis* (=*T. colubriformis*). According to Mönnig (*10*), who later (*11*) described the clinical symptoms and gross pathology of this disease as it occurred in South Africa, it was not until 1923 that these small trichostrongyles were recognized as the definite cause of serious losses among sheep in that part of the world. At about the same time, the nematodes in question were also suspected of causing severe losses among sheep in California.[3] In 1932 Boughton (*4*) described typical symptoms of trichostrongylosis in connection with an outbreak of the disease in goats in Texas, and in 1937 Schmidt (*12*) reported briefly on unsuccessful attempts to treat the disease. That trichostrongylosis is also of considerable economic importance in Australia has been shown by Edgar (*5*), Kauzal (*9*), Bennetts (*3*), and Gordon and Ross (*8*), who studied various clinical aspects of the disease, and by Seddon and Ross (*13*), Belschner and Edgar (*2*), Gordon (*7*), and Gordon and Ross (*8*), who worked on the treatment for it. So far as the writer is aware, the paper by Gordon and Ross is the only one containing a record of the production of fatal cases of trichostrongylosis by the administration of infective larvae to susceptible host animals.

The purpose of the present paper is to report observations on cases of trichostrongylosis produced in host animals raised in cages relatively free from parasites and experimentally infected with known numbers of infective larvae of *Trichostrongylus* spp.

EXPERIMENTAL METHODS

Eleven cross-bred Hampshire-Southdown lambs and three Saanen kids, about 6 months old, were used in this investigation. These host animals were born at the Agricultural Research Center, Beltsville, Md. They were kept at all times in cages having concrete floors, which were cleaned daily to prevent infestation with parasites other than those administered.

[1] Received for publication November 3, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 769.

[3] Personal communication by the late M. C. Hall, formerly Chief of the Zoological Division, Bureau of Animal Industry.

The experiments involving lambs 57, 58, 68, 70, 72, 122, and 61 were conducted in 1935. The first five lambs of this group received larvae of *Trichostrongylus colubriformis*, whereas lamb 61 remained uninfected and served as a control. The experiments involving lambs P2, 112, 117, and 123 and kids 127, 130, and 131 were conducted in 1937. These animals received larvae of *T. vitrinus*, *T. colubriformis*, and a small number of the larvae of *T. axei*.

To obtain infective larvae, specimens of the nematodes desired were removed from infested sheep at the time of slaughter, and the females of each species were isolated and cut up with a pair of fine scissors. The eggs thus liberated were cultured at room temperature for about 7 days in sterile sheep feces and animal charcoal. The infective larvae were removed from the cultures by means of the Baermann apparatus and suspended in water. They were then administered by means of a funnel and rubber tube to parasite-free lambs, which later served as sources of infective material for the experiments. When these lambs began to pass trichostrongyle eggs, the feces were cultured as indicated above, and the infective larvae obtained were administered to the experimental animals as shown in tables 1 and 2.

The progress of the infestations was followed by the use of standard egg-counting methods, and the data thus obtained were recorded in terms of the number of eggs per gram of feces. The general appearance of the animals and the consistency of the feces were noted from day to day. Samples of blood were taken from all the animals at frequent intervals, and the total number of red and white cells per cubic millimeter of blood, the amount of hemoglobin per 100 cc, and the cell-volume percentage were ascertained. Blood smears were stained with Wright's stain, and the percentages of the different types of white cells were recorded.

Post-mortem examinations were made of all animals used in the experiments; the entire alimentary tract was removed and examined for parasites and lesions. When the post-mortem examination was made soon after death, pieces of the viscera were fixed in Bouin's solution, embedded in paraffin, and submitted to the Pathological Division of the Bureau of Animal Industry for histological examination.

DATA OBTAINED

The number of infective larvae administered to the animals that survived the infestation and the number of trichostrongyles recovered post mortem from these animals are recorded in table 1. Similar data for the host animals that died as a result of the infestation are given in table 2. No nematodes were recovered from the control lamb (No. 61).

TABLE 1.—*Data on host animals with nonfatal infestations of Trichostrongylus spp.*

Animal species and No.	Dates on which larvae were administered	Larvae per dose	Doses administered	Total larvae administered	Trichostrongyles recovered post mortem	
	[1] *1935*	*Number*	*Number*	*Number*	*Number*	*Percent*
Lamb 57	July 8, Aug. 12	250	36	112,750	7,004	6.212
	Aug. 20, Aug. 26	500	7			
	Aug. 27, Oct. 6	250	41			
	Oct. 7, Nov. 10	5,000	18			
Lamb 68	Mar. 13	250	1	138,250	8	.006
	Apr. 17	1,500	1			
	Apr. 24	3,000	1			
	May 17	7,500	1			
	July 9, Aug. 12	1,000	35			
	Aug. 20, Aug. 26	2,000	7			
	Aug. 27, Nov. 11	1,000	77			
Lamb 70	July 9, Aug. 12	500	35	63,000	4	.006
	Aug. 20, Aug. 26	1,000	7			
	Aug. 27, Nov. 11	500	77			
Lamb 122	Nov. 8	80,000	1	80,000	([2])	([2])
	1937					
Lamb 123	June 24	([3])	1	[4] 111,250	([2])	([2])
	July 27	1,100	1			
	Aug. 2	1,200	1			
	Aug. 11	2,500	1			
	Aug. 17	1,450	1			
	Nov. 4	15,000	1			
	Dec. 15	90,000	1			
Lamb P2	Nov. 8	100,000	1	100,000	0	.000
Total			353	605,250	7,016	
Average		1,715	58.8	100,875	1,754	[5] 1.556

[1] Administered daily for periods shown.
[2] No post-mortem examination made.
[3] Unknown.
[4] Exclusion of number administered June 24.
[5] Average for 4 lambs.

TABLE 2.—*Data on host animals with fatal infestations of Trichostrongylus spp.*

Animal species and No.	Dates on which larvae were administered	Larvae per dose	Doses administered	Total larvae administered	Trichostrongyles recovered post mortem	
	[1] *1935*	*Number*	*Number*	*Number*	*Number*	*Percent*
Lamb 58	July 8, Aug. 12	5,000	30	440,000	117,756	26.46
	Aug. 20, Aug. 26	10,000	7			
	Aug. 27, Oct. 3	5,000	38			
Lamb 72	Sept. 16, Oct. 12	5,000	27	135,000	63,875	47.31
	1937					
Lamb 112	Nov. 19	150,000	1	150,000	83,678	55.79
Lamb 117	Oct. 22	115,000	1	115,000	80,178	69.72
Kid 127	Oct. 12	50,000	1	100,000	10,889	10.89
	Nov. 8	50,000	1			
Kid 130	Sept. 25	33,000	1	78,000	52,626	67.47
	Sept. 27	45,000	1			
	Oct. 8	[2] 300,000				
Kid 131	Aug. 30, 31; Sept. 3, 10	3,000	4	196,000	29,917	15.26
	Aug. 28; Sept. 2, 4, 11, 13, 15, 16, 17, 20, 22, 23.	6,000	11			
	Sept. 9, 18	12,000	2			
	Sept. 27	14,000	1			
	Oct. 6	80,000	1			
Total			133	1,214,000	438,919	
Average		9,128	19.0	173,429	62,703	41.84

[1] Administered daily for periods shown.
[2] Not included in total for reason stated in text.

The essential points brought out by the data in tables 1 and 2 may be summarized as follows: The smallest number of larvae producing death in a kid was 78,000, whereas the smallest number producing death in a lamb was 115,000. In computing the totals, the dose of 300,000 larvae administered to kid 130 on October 8, was not included since these larvae were given only 2 days before the onset of diarrhea and were therefore not considered to be responsible for the appearance of symptoms or the death of the animal. The results indicate that the kids used were somewhat more susceptible to *Trichostrongylus* infestation than the lambs. Further evidence in support of this finding was obtained from two pairs of experimental animals. Kid 130 and lamb 122 received 78,000 and 80,000 larvae, respectively, and kid 127 and lamb P2 each received 100,000 larvae, but only the kids succumbed to the infestation. The source of the infestations in the four animals was identical.

The repeated administration of small doses of larvae to the experimental animals apparently enabled them to build up some resistance and to survive the infestation. This phenomenon is illustrated by lambs 57, 68, 70, and 123. Evidence that resistance developed in the surviving animals is shown by the fact that only 1.556 percent of the larvae administered were recovered from these animals post mortem, whereas 41.84 percent were recovered from animals that died from the infestation. The average number of larvae administered to the latter group was only 1.7 times the number administered to the former.

In the case of repeated doses of larvae, if the smallest dose was 3,000 or more, the acquired resistance built up was not sufficient to protect the animal, as it developed typical symptoms of trichostrongylosis and died as a result of the infestation. This phenomenon is illustrated by data obtained for lambs 58 and 72 and kid 131, shown in table 2. These results are in general agreement with those of Gordon and Ross (*8*), who reported that daily doses of 4,000 trichostrongyle larvae were fatal to their experimental lambs.

The clinical observations made on the infested animals are recorded in table 3. As is shown in this table, the eggs first appeared in the feces of the infested animals from 17 to 21 days after infection. Host animals 112, 117, and 130, for which data on the first appearance of eggs are lacking, developed symptoms of trichostrongylosis and died before worm eggs were detected in the feces. In these three cases a positive diagnosis of trichostrongylosis could not have been made prior to post-mortem examination. That these three animals undoubtedly died of trichostrongylosis is shown by the large number of trichostrongyles recovered post mortem (table 2). Mönnig (*10, 11*) reported that the eggs of *Trichostrongylus* spp. appeared in the feces of infected sheep 21 days after infection. In the present experiments, however, eggs appeared in the feces 17 days after infection, which period is similar to that required for *Haemonchus contortus* and *Cooperia curticei*.

TABLE 3.—*Clinical observations on animals infected with Trichostrongylus spp.*

Cases, animal species, and No.	Date of first administration of larvae	Interval between first dose of larvae and appearance of eggs in feces	Maximum eggs per gram of feces	Interval between first dose of larvae and maximum number of eggs in feces	Interval between first dose of larvae and appearance of diarrhea	Date of death	Interval between first dose of larvae and death	Interval between first appearance of diarrhea and death
Nonfatal:		*Days*	*Number*	*Days*	*Days*		*Days*	*Days*
Lamb 57	July 8, 1935	21	1,800	64				
Lamb 68	Mar. 13, 1935		4,600	81				
Lamb 70	July 9, 1935	20	2,600	49				
Lamb 122	Nov. 8, 1935	19	8,200	21				
Lamb 123	June 24, 1937	18						
Lamb P2	Nov. 8, 1937		5,800	36	22			
Fatal:								
Lamb 58	July 8, 1935	17	5,000	29	35	Oct. 4	88	53
Lamb 72	Sept. 16, 1935	17	9,400	38	26	Nov. 16	61	35
Lamb 112	Nov. 19, 1937				14	Dec. 10	21	7
Lamb 117	Oct. 22, 1937				13	Nov. 7	16	3
Kid 127 [1]	Oct. 12, 1937		2,800	32	59	Dec. 23	72	13
Kid 130 [1]	Sept. 25, 1937				15	Oct. 13	18	3
Kid 131	Aug. 28, 1937	19	[2] 54,000	63	44	Oct. 30	63	19
Average		18.7	5,025	45.89	28.5		48.43	19.00

[1] Destroyed while in a moribund condition.
[2] Not included in average, for reasons stated in text.

The maximum number of eggs per gram of feces appeared from 21 to 81 days after the first administration of larvae. The time necessary for the maximum number of eggs to appear, as well as that necessary for diarrhea to develop and for death to occur, was associated with the severity of the infestation and the rapidity with which it was acquired. These intervals were short following a single large dose or a few large doses of larvae, but were longer following small doses of larvae administered over a relatively long period. The exceptionally high maximum egg count per gram of feces for kid 131 was obtained on the day the animal died. Since the host animal had not eaten its full ration for a few days prior to death, the quantity of feces passed was considerably reduced. As a result of this decrease in the feces, the increase in number of eggs per gram of feces was out of all proportion to the actual number of eggs produced by the worms under normal conditions. Consequently, in computing the average maximum egg counts shown in table 3, the figure obtained for kid 131 was omitted.

The maximum number of eggs per gram of feces in fatal cases of trichostrongylosis varied from 1,800 to 54,000 (table 3). It is obvious that no eggs are to be found in feces of infested animals if symptoms and death occur before the worms mature, whereas a large number of eggs per gram of feces may be found if the quantity of feces expelled is less than normal. Kauzal (*9*) found that the number of eggs never exceeded 3,600 per gram of feces in the cases of trichostrongylosis that he studied. However, he quotes Gordon as reporting egg counts as high as 20,600 eggs per gram of feces. These quite different observations appear to be easily explained by the present findings.

Diarrhea, which is characteristic of trichostrongylosis, occurred 13 to 59 days after the first administration of larvae. It appeared suddenly and in most cases was continuous until the death of the host. However, there were two exceptions. Lamb P2 developed diarrhea that

lasted for 3 days only, beginning on November 30; thereafter the animal showed no further symptoms. Kid 127 also developed a transient diarrhea on December 10, which disappeared after 6 days but recurred on December 21. At this time the diarrhea was profuse and continuous, and the animal became so weak that it was killed on December 23. Gordon and Ross (*8*) reported that diarrhea occurred in their experimental lambs 6 to 7 weeks after daily infection with 4,000 larvae was begun. The variation in length of the period observed in the present experiments may be accounted for by the different rate of infestation to which the animals were submitted.

In connection with diarrhea occurring in lambs affected with trichostrongylosis, Edgar (*5*) and others have mentioned the very dark green, almost black, color of the feces, from which the disease gets its common name "black scours." The diarrheic feces observed during the present investigation were not dark enough to be called black. Since little blood was ever found in the feces of the experimentally infected animals, it is probable that the black color reported by other workers may have been due to the green grass ingested by the animals or to the presence of blood in the feces caused by an accompanying infestation with *Haemonchus contortus*.

Without exception, the animals that harbored fatal infestations of *Trichostrongylus* appeared depressed, suffered from abdominal pain, and were unable to eat all the feed given them. Figure 1, *A*, shows a lamb in the last stages of trichostrongylosis; the dejected appearance, emaciated condition, and soiled hindquarters of the animal and the liquid feces on the floor of the cage (see arrow) may be noted. The emaciation of this lamb and of lamb 72, the carcass of which is shown in figure 1, *D*, was due to the inability of these animals to eat sufficient feed during the period of infestation to keep them in good nutritional condition, and to dehydration of the tissues brought about by the continuous diarrhea. In animals dying of trichostrongylosis there was no evidence of anemia, but an examination of the blood showed a marked increase in the number of leucocytes and in the percentage of polymorphonuclear neutrophiles. Andrews (*1*) also found no anemia in sheep infected with *Cooperia curticei*, and Fourie (*6*) reported a neutrophilic leucocytosis in fatal cases of haemonchosis.

Death occurred from 16 to 88 days after the first administration of larvae and from 3 to 53 days after the onset of diarrhea. Shortly before death, the animals became rapidly weaker, went into a state of collapse, and died about 2 hours later (fig. 1, *B* and *C*.)

Bennetts (*3*) reported that animals suffering from trichostrongylosis died of malnutrition resulting from loss of appetite and failure to ingest sufficient feed. In the present investigation it was found that although there was a reduction in the quantity of feed ingested by most of the animals, the severe emaciation associated with malnutrition was noted only in the animals that survived for a relatively long period. If an animal lived only a short time after infection, it reacted very much like the others, becoming weak, collapsing, and dying a few hours later. Severe emaciation occurred in the two lambs that received their infestation from day to day in a manner similar to that in which it would have been acquired from pasture. The remaining animals succumbed too quickly for a long-drawn-out process, such as starvation, to operate.

The number of worms recovered post mortem from fatal cases of trichostrongylosis was variable, as shown in table 2. This was also found to be true by Kauzal (*9*), who reported finding 35,000 to 40,000 worms commonly, and as many as 83,000 in one case. He also stated that Gordon had found one lamb that harbored 21,400 worms and another that harbored 26,200. Gordon and Ross (*8*), however, reported only 3,900 to 11,360 worms from their lambs that died of experimental trichostrongylosis.

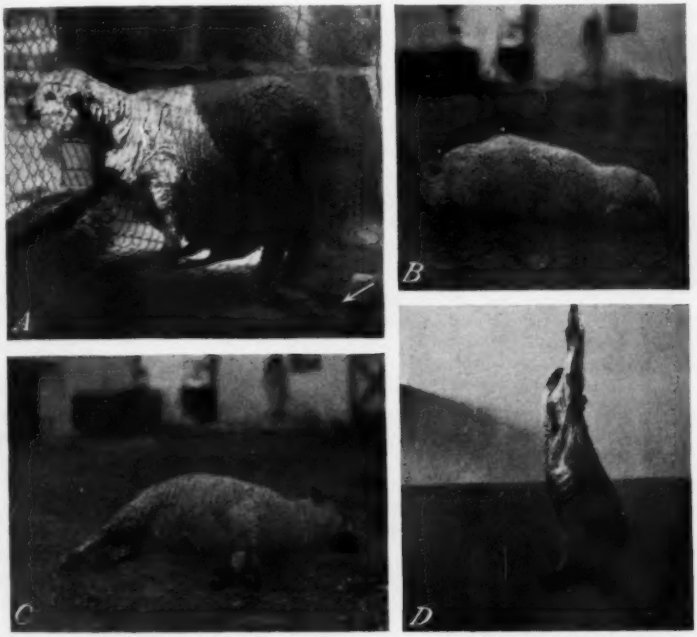

FIGURE 1.—Lamb 58: *A*, The day before it was found dead of trichostrongylosis; *B*, too weak to stand—typical of last stages of trichostrongylosis; *C*, just before death from trichostrongylosis. *D*, Carcass of lamb 72 after death from trichostrongylosis.

The following pathological changes were noted on post-mortem examination of the seven animals that died of trichostrongylosis. Serous fluid was found in the abdominal cavity, and in one case (kid 131) the mesentery was edematous. In all cases the liver was friable, and the gall bladder was distended with bile. The contents of the gall bladder of kid 127 were gelatinous and the outer surface of the distal portion of the organ was covered with a fibrinous exudate. There was diffuse enteritis throughout the small intestine in all animals, and in lamb 117 there were petechial hemorrhages in the intestinal mucosa. The pyloric region of the abomasum of kids 127 and 131 also showed some inflammation.

Edgar (*5*) stated that the outstanding feature seen on post-mortem examination was the extremely emaciated condition of the carcass. He also mentioned the absence of anemia, the presence of serous fluid in the abdomen, and the absence of lesions from the small intestine or, at most, the occurrence of minute ecchymotic areas. Boughton (*4*) reported that the mucous membrane of the entire length of the small intestine was studded with small ulcers. Mönnig (*11*) also reported the friable condition of the liver, and in addition he observed a jelly-like swelling under the throat of animals suffering from trichostrongylosis. Such a swelling was observed in kid 127 but in none of the other animals.

The histological examination of the lungs, spleen, intestine, liver, and kidneys pointed to the existence of a generalized circulatory disturbance, which had given rise to chronic passive congestion, accompanied by degenerative changes in the liver and kidneys, together with some infiltration of these areas with polymorphonuclear leucocytes. The relationship between the worm infestations, the diarrhea produced by the parasites, and the histological findings are difficult to explain on the basis of available data.

SUMMARY

In the present experiment 11 cross-bred Hampshire-Southdown lambs and 3 Saanen goat kids about 6 months old were used. The work was carried on at the Agricultural Research Center, Beltsville, Md., in 1935 and 1937.

The data show that the infestation of sheep and goats with nematodes of the genus *Trichostrongylus* usually produces a profuse, continuous diarrhea, the feces containing little or no blood. Trichostrongylosis can usually be diagnosed by the finding of the eggs of *Trichostrongylus* spp. in the feces of the infected animals. However, three experimental cases were observed in which the worms had not yet reached egg-laying maturity at the time of death of the host animals.

Under the conditions of the experiments, the smallest total number of larvae producing death in a kid was 78,000, whereas the smallest total number in a lamb was 115,000. This finding, together with the additional observation that lambs but not goats were able to survive a dose of 100,000 larvae, indicates that goats are probably more susceptible than sheep to trichostrongyle infestations.

Repeated administration of small doses of larvae to susceptible hosts enabled the hosts to build up sufficient resistance against the infestation to survive. This resistance is shown by the fact that only about 1.5 percent of the larvae established themselves and were recovered post mortem from the host animals that survived the infection, whereas nearly 42 percent were recovered from the animals that died from the infection. If the smallest dose of the series was 3,000 or more larvae, the resistance built up by the host animal was not sufficient to protect it, and death followed.

The eggs of these nematodes appeared in the feces after a minimum period of 17 days. The maximum number of eggs per gram of feces was found 21 to 81 days after the first administration of larvae and varied greatly in different animals. These observations show that the number of eggs per gram of feces in fatal cases of trichostrongylosis

may vary from 0 to 54,000. It is obvious that no eggs are to be found in the feces of infested animals if symptoms and death occur before the worms mature, whereas a large number of eggs per gram of feces may be found if the quantity of feces expelled is less than normal.

Diarrhea appeared 13 to 59 days after the first infection. The variation in the length of this period, as well as that for the appearance of the maximum number of eggs in the feces and the occurrence of death, was associated with the severity of the infestation and the rapidity with which it was acquired. These periods were shorter after a single heavy infestation and longer after the administration of many small daily doses of larvae.

There was no anemia in animals dying of trichostrongylosis, but there was an increase in the number of white cells per cubic millimeter.

Although there was a reduction in the quantity of feed ingested by most of the animals, severe emaciation associated with malnutrition was noted only in those animals that survived for a relatively long period. The emaciation was apparently due not only to partial starvation but also to the dehydration of the tissues brought about by the diarrhea from which these animals suffered.

Death of the animals occurred 16 to 88 days after the first infection and 3 to 53 days after the appearance of diarrhea.

The number of worms recovered from the animals dying of trichostrongylosis ranged from 10,889 to 117,756. No extensive lesions in the intestine were found, but serous fluid in the abdomen, diffuse enteritis of the small intestine, and a friable condition of the liver were noted. A swelling was observed under the throat of one kid but not in any of the other animals.

Histological examination of the tissues taken from animals that died of trichostrongylosis indicated that death was caused by a generalized circulatory disturbance, which had given rise to chronic passive congestion accompanied by degenerative changes in the liver and kidneys. These areas were infiltrated with polymorphonuclear leucocytes.

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LIFE HISTORY OF THE NEMATODE COOPERIA CURTICEI, AND DEVELOPMENT OF RESISTANCE IN SHEEP[1]

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INTRODUCTION

Cooperia curticei, a nematode parasitic in the small intestine of sheep and goats, is of more or less cosmopolitan distribution. Curtice (*5*)[2] first reported this parasite from sheep, under the name *Strongylus ventricosus* Rud. Ransom (*28*) created the genus *Cooperia* for a group of nematodes having an anterior cephalic cuticular swelling, a lyre-shaped dorsal ray, and no gubernaculum, and selected as the type species of this genus *Strongylus curticii* Giles 1892 (=*S. ventricosus* Curtice 1890). Curtice (*5*) expressed the opinion that this nematode was of little or no importance as a cause of parasitic disease. Edgar (*9*), however, reported fatalities in goats that he believed to be the result of heavy infestations with this parasite. Ransom (*30*) and Hung (*13*) described lesions in the intestine of cattle caused by another species of *Cooperia*, *C. punctata;* and Schwartz (*36*, *37*) and Gomez (*12*) described lesions in the intestine of a carabao caused by *C. nodulosa*. Since no detailed study of the life history of *C. curticei* appears to have been made, and since, so far as the writer has knowledge, infestations with this nematode have not been previously associated with the formation of nodules in the intestinal mucosa of the host, the following data are made available in the literature. The investigation into the life history of *C. curticei* was carried out at the Agricultural Research Center, Beltsville, Md., during 1934, 1936, and 1937.

MATERIALS AND METHODS

Specimens of *Cooperia curticei* were collected from the small intestines of sheep slaughtered at an abattoir in Washington, D. C. The female nematodes were carefully washed in physiological saline solution until they were entirely free from debris and were then cut up with a pair of fine scissors. The eggs thus liberated were cultured in a mixture of helminthologically sterile sheep feces and granular bone charcoal moistened with distilled water. This mixture was put into Petri dishes 20 cm in diameter. The lids were placed on the dishes, which were left at room temperature for about 7 days. The infective larvae that developed from the eggs were then removed from the cultures by means of the Baermann apparatus, suspended in water, and administered by mouth to a lamb by means of a funnel and rubber tube. The lamb had been raised in a cage and was relatively free from nematode parasites at the time. After infection this lamb was kept as a source of material for the further study of the parasite.

[1] Received for publication November 8, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 783.

For the study of the development of the embryos and newly hatched larvae, eggs obtained from freshly passed feces suspended in water were pipetted into small watch glasses containing distilled water. The development of the embryos was followed under the low power of a compound microscope, and those in a particular stage of development were removed and studied in detail with the oil-immersion objective.

For the study of the preparasitic stages, larvae were removed from the charcoal-feces cultures at various intervals and a record made of their development at the time of observation.

In the study of the parasitic stages seven lambs were used. They were born at the Agricultural Research Center, and were less than 1 year old at the beginning of the experiment. The animals were raised in cages similar to the one in which the lamb that served as the source of infection was kept. All the lambs except three were relatively free from parasites when the experiment began. Two of these three had been fed pasture grass for several weeks prior to the experiment, and one had been on pasture during the preceding summer. Large numbers of infective larvae of *Cooperia curticei* were administered to the experimental lambs. The lambs were killed at different intervals after the administration of the larvae, and the stage of development of the parasites recorded. The presence of other nematode parasites in three of the lambs did not interfere with the present experiment since the developmental stages of *C. curticei* could be easily separated from those of the other species and from the more mature specimens of *C. curticei* that were found in these animals.

Since the life histories of nematodes closely related to *C. curticei* have been studied by Veglia (*43*), Monnig (*24*), and Threlkeld (*42*), certain details of the morphology and development observed during the present investigation that were found to be similar to those reported by the authors mentioned have been omitted from this paper.

For obtaining data on resistance of lambs to superinfection, seven animals were used. Lambs 61, 63, 65, 88, 102, and 118 were 4 to 8 months of age at the beginning of the experiment and had not previously been infected with *C. curticei*. Lamb 103, however, had been infected with this nematode during the previous summer, and was about 17 months old at the time the doses of larvae indicated in table 1 were administered. Since this lamb was used only as a source of infective material during the previous summer, no exact record of the infection was kept during that time.

LIFE HISTORY OF COOPERIA CURTICEI

PREPARASITIC STAGES

The eggs of *Cooperia curticei* (fig. 1, *A*), are provided with thin, transparent shells, with equal poles, and with sides more or less parallel. They vary from 70μ to 82μ in length and from 35μ to 41μ in width. The average length and width of 102 eggs were 76.8μ and 37.8μ, with a mean standard deviation of ± 0.024 and ± 0.015, respectively. When passed in the feces, the egg contains an embryo in the morula stage, having 16 to 32 cells.

When cultured at room temperature the eggs hatch in about 20 hours after being passed in the feces. The newly hatched rhabditi-

form larvae (fig. 1, *B*) are approximately 300μ long and grow to a length of about 500μ. The larvae enter the first lethargus about 12 hours after hatching. They become active again and cast their sheaths approximately 18 hours later, or about 30 hours after hatching.

The second-stage larvae (fig. 1, *C*) are similar to those of the first stage except that they are larger and the intestinal cells contain more

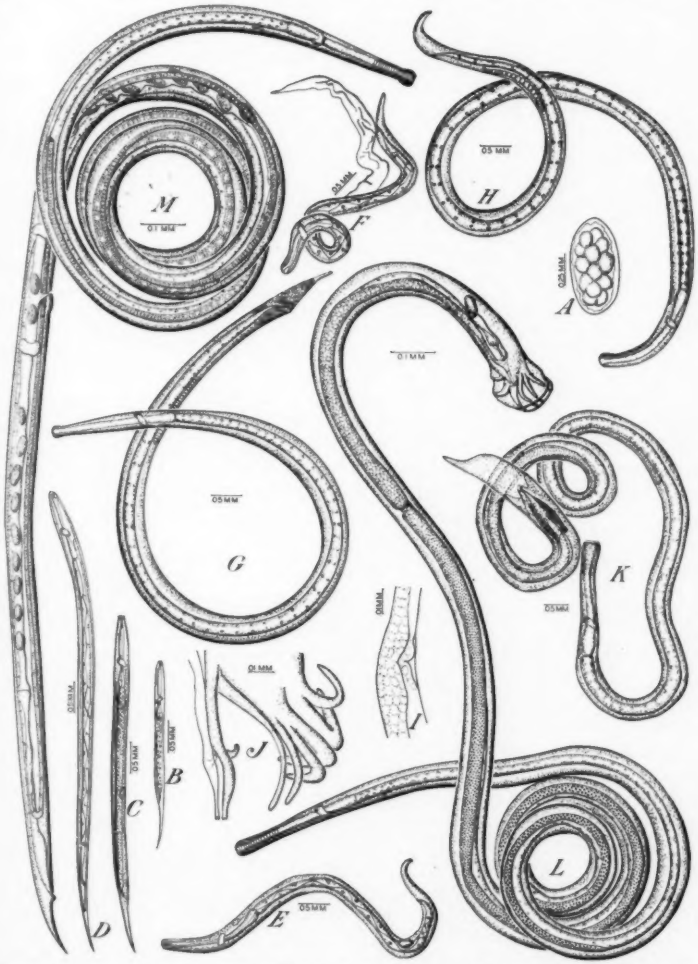

FIGURE 1.—Stages in the life history of *Cooperia curticei: A*, Egg; *B*, first-stage larva; *C*, second-stage larva; *D*, third-stage infective larva; *E*, third-stage parasitic larva; *F*, third-stage parasitic larva undergoing third ecdysis; *G*, fourth-stage larval male; *H*, fourth-stage larval female; *I*, formation of vagina from cells in body wall of female; *J*, bursal rays of fourth-stage larval male; *K*, larval male just after fourth ecdysis; *L*, adult male; *M*, adult female.

granules. The larvae were found to feed actively for approximately 48 hours before entering the second lethargus, which lasts about 12 hours.

About 90 hours after hatching, the larvae become active again. They have now reached the infective stage and are enclosed in the cuticle of the second stage. They measure 711μ to 860μ in length, including the sheath (fig. 1, *D*). This stage of *C. curticei* has been described previously by Dikmans and Andrews (*6*). Later, Andrews (*2*) published a figure showing that the third-stage larva of this species is characterized by having a band of fibers surrounding the buccal capsule. This observation, however, has been found to be incorrect, the bands shown in figure *B* of that paper being the transverse striations of the cuticle.

The exsheathing of the third-stage larvae was observed to take place in a very dilute alkaline solution of sodium hypochlorite (antiformin), as was previously noted by Lapage (*20*), who first reported the exsheathing of the infective larvae of other species of trichostrongyles in this solution.

PARASITIC STAGES

Two days after the experimental lambs had been infected, all the larvae recovered from the small intestines had completed the second ecdysis and appeared to be actively feeding in the lumen of the gut. They were still in the third stage and showed signs of the approaching third ecdysis (fig. 1, *E*). The lips and papillae of the fourth-stage larvae could be seen forming at the anterior end of the esophagus, which had become much thicker than that of the third-stage preparasitic larvae. The larvae appeared to increase very little in length during the parasitic part of the third stage, some of them being shorter than the infective larvae. The genital primordium had moved posteriorly, and the posterior ends of the larvae of both sexes were curved dorsally.

Three days after infection the larvae were still in the third stage, and many of them were found to be migrating into the crypts between the villi of the anterior portion of the small intestine. A large number of sections of intestinal wall were examined microscopically, and numerous larvae were seen in the crypts on the third, fourth, and fifth days after infection (fig. 2, *A*), but in no instance were they found to penetrate the mucosa.

During the fourth day after infection the larvae in the crypts passed through the third ecdysis (fig. 1, *F*) and immediately thereafter the larvae, now in the fourth stage, began to grow rapidly. Their anterior ends remained in the crypts, whereas their posterior ends protruded from between the villi into the lumen of the intestine. At this time there was great variation in the size of the larvae, those having recently passed the third ecdysis being much smaller than those somewhat older. The smallest fourth-stage larva recovered was 650μ long, but most of them were about 1.4 mm long. Sex differentiation was possible at this stage, the posterior end of the larval male being shorter and thicker than that of the larval female. The genital primordium of the female was found to be nearer the posterior end than that of the male.

By the fifth day after infection most of the larvae had returned to the lumen of the intestine. They now measured 1.6 mm in length.

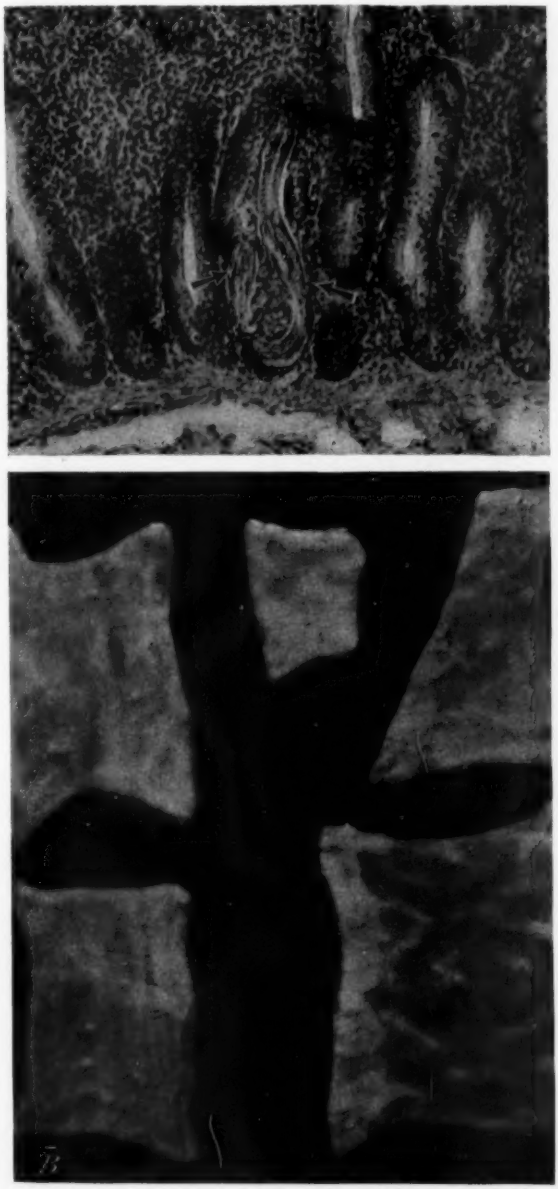

FIGURE 2.—*A*, Larvae (*see* arrows) of *Cooperia curticei* in a crypt of the intestinal mucosa of a nonresistant lamb; *B*, section of intestine showing nodules in which larvae of *C. curticei* were found.

The genital primordium of both sexes had increased in length. In the larval male (fig. 1, *G*), the genital primordium was about 260μ from the posterior end and differed from that of the female in having both germ cells at the anterior pole. In the larval female (fig. 1, *H*), the genital primordium was situated about 180μ from the posterior end and consisted of a double row of epithelial cells with a germ cell at either end. At this stage the sexes could be easily distinguished by the increased thickness and bluntness of the posterior end of the male and by the slender, pointed, posterior end of the female.

Six days after infection the larvae measured about 1.8 mm in length. In some specimens certain regions of the genital primordium had become differentiated so that the parts which form the ovaries, uterus, and ovejectors of the female, and the testis, seminal vesicle, and sperm duct of the male, could be distinguished. In the vulvar region of the female, cells could be seen in the body wall that ultimately connected with the primordium of the ovejectors to form the vagina of the adult (fig. 1, *I*). In a few specimens the lumen of the ovejectors was visible. The sperm duct of the male had not yet become connected with the cloaca. The posterior ends of the males were bilobed and the genital cone was in the process of formation. In a few specimens the spicules and bursal rays were beginning to form.

Eight days after infection the larval males were 2.4 mm long and most specimens were approaching the fourth, or final, ecdysis as shown by the loosened cuticle of the fourth stage that enclosed the larvae. The sperm duct was now connected with the cloaca, and the testis had advanced anteriorly almost half way to the base of the esophagus. Although many of the males were nearing maturity, others showed great variation in the spicules and bursa.

The rays of the bursa of a fourth-stage larval male are shown in figure 1, *J*. These rays were found to develop in a manner similar to those of *Haemonchus contortus*, *Monodontus trigonocephalus*, and *Gaigeria pachyscelis*, as reported by Veglia (*43*), Cameron (*4*), and Ortlepp (*27*), respectively. The formation of the dorsal ray by the fusion of the two adjacent dorsal branches of the longitudinal bands of the two sides in *Cooperia curticei* agrees with that reported as occurring in *G. pachyscelis* and *M. trigonocephalus*.

The larval females at this stage were about 2.9 mm long. In some specimens muscle cells could be seen in the walls of the ovejectors, which had a distinct lumen. The lumen of the vagina now connected with that of the ovejectors. The primordia of the ovaries had increased in length. The posterior ovary had already turned anteriorly after having reached the posterior limit of its development, whereas the anterior ovary had reached about half way to the base of the esophagus.

Nine days after infection, when the larvae had attained a length of about 3 mm, the fourth ecdysis occurred in the lumen of the small intestine (fig. 1, *K*). After this ecdysis the young adults matured rapidly.

Ten days after infection most of the worms had passed the fourth ecdysis. The genital system of both sexes was still immature, as shown by its size as compared with that of the adult worms, by the light-colored spicules of the males, and by the absence of eggs in the genital system of the females.

Twelve days after infection both males and females were approaching maturity, as shown by the growth of the genital system, by the darkening of the spicules of the males, and by the formation of eggs in the ovaries and uteri of the females.

Fourteen days after infection the worms had reached the size of mature individuals. The males (fig. 1, *L*) averaged about 5.3 mm and the females (fig. 1, *M*) about 6.5 mm long, respectively. The adults of this species have been described in detail by Ransom (*29*). A redescription, therefore, is unnecessary.

Fifteen days after infection the eggs were found in the feces of infected lambs.

DISCUSSION

In general, the life history of *Cooperia curticei* as reported in the present paper was found to be similar to those of the closely related trichostrongyles, namely, *Haemonchus contortus*, reported by Veglia (*43*); *Ostertagia circumcincta*, reported by Threlkeld (*42*); and *Trichostrongylus* spp., reported by Monnig (*24*). However, certain differences were observed.

The free-living phases of the life cycles of all these trichostrongyles are practically identical, since the variations reported could be attributed to the different conditions under which the larvae were cultured. However, other observed differences could not be so readily explained.

According to data reported by Monnig (*24*), the larvae of *Trichostrongylus* spp. appear to require a longer time to reach the third ecdysis than do those of *Haemonchus contortus*, *Ostertagia circumcincta*, and *Cooperia curticei*. This difference in the time necessary for development may explain Monnig's observation that the larvae of *Trichostrongylus* spp. increased in length during this stage of development. The larvae of *C. curticei* were found to undergo little if any increase in length during this stage, and in this respect they were similar to the larvae of *H. contortus* and *O. circumcincta*.

Unlike the larvae of *Haemonchus contortus* and *Ostertagia circumcincta*, which penetrate the mucosa of the abomasum in preparation for the third ecdysis, the larvae of *Cooperia curticei* were found to migrate superficially into the crypts of the intestinal mucosa, where they undergo the third molt. This observation was in keeping with that of Monnig, who reported a similar behavior of the larvae of *Trichostrongylus* spp. This similarity is not surprising since *Cooperia* is so closely related to *Trichostrongylus*.

According to Monnig's data, the larvae of *Trichostrongylus* spp. apparently required about 36 hours longer than the larvae of *Haemonchus contortus* and *Cooperia curticei*, and about 60 hours longer than the larvae of *Ostertagia circumcincta*, to reach the fourth ecdysis. The first-mentioned species also required 7 to 8 days longer than the period necessary for the other three nematodes to reach maturity. It has been the writer's experience, however, that under similar conditions *Trichostrongylus colubriformis* matures in about the same length of time as the other three species, the eggs being found in the feces of lambs 17 days after experimental infection.

Alicata (*1*) reported that the sex of the preparasitic larvae of the trichostrongyle *Hyostrongylus rubidus* could be ascertained by noting the relative position of the genital primordium and the fourth genital

giant cell. An attempt to confirm this finding during the present study was unsuccessful. Sex differentiation in *Cooperia curticei* was not apparent until the end of the third stage, this finding being in accordance with the observations on this point made by Veglia (*43*) and Monnig (*24*) on *Haemonchus contortus* and *Trichostrongylus* spp., respectively.

DEVELOPMENT OF ACQUIRED RESISTANCE TO SUPERINFECTION WITH COOPERIA CURTICEI

EXPERIMENTAL RESULTS

During the course of the experimental infection of lambs with *Cooperia curticei*, it was noted that the number of adults recovered from the small intestine after administration of a given number of larvae by mouth varied greatly in individual host animals. It was also noted that in the host animals in which relatively few adults were found, there occurred a tissue reaction that had not previously been described as associated with infections with this parasite. This reaction was first observed at the post-mortem examination of experimentally infected lambs in 1934. It was evidenced by the presence of macroscopic nodules in the mucosa of the small intestines of the infected lambs similar to those illustrated in figure 2, *B*.

A number of these nodules were sectioned and found to contain nematode larvae. Although the larvae could not be directly identified, they were undoubtedly those of *C. curticei*, since the experimental animals had received thousands of the infective larvae of this species and had been prevented from acquiring larvae of other species. Stained sections of the nodules were submitted to the Pathological Division of the Bureau of Animal Industry for histological examination. The report on the nodules was as follows:

> The small parasitic nodules were located in the deeper structures of the intestinal mucosa, practically in contact with the basement membrane. So far as could be determined from the sections examined, the cellular elements toward the periphery of the nodules were largely lymphoid in nature, mixed with plasma cells and a few large mononuclears, whereas toward the centers, particularly of the older nodules, a larger percentage of the cells were polymorphonuclear leucocytes. Also, in some of the older nodules there was proliferation of the connective-tissue elements with a slight tendency to encapsulation of the lesions. In some of the sections there was a partial palisade formation of the connective-tissue cells at the borders of the necrotic centers and a limited number of partially degenerated giant cells were noted in a number of the nodules. Portions of the nematode larvae could be seen in the necrotic centers of a number of the older nodules.

During the examination of numerous sections of the intestine of lambs infected with *Cooperia curticei*, some larvae were found free in the crypts of the mucosa as shown in figure 2, *A*, whereas others were surrounded by various degrees of tissue reaction. The latter appeared to include all stages of nodule development, from a larva within a crypt surrounded by a few polymorphonuclear leucocytes to the formation of a complete nodule. Figure 3, *A* to *D*, inclusive, illustrates the probable formation of the nodules as postulated from these observations. According to the data obtained from a study of the slides examined, nodule formation begins with an accumulation of polymorphonuclear leucocytes about the larvae in the crypts, much as in any inflammatory process (fig. 3, *A*). The number of leucocytes increases and, together with the necrosis of tissue in the

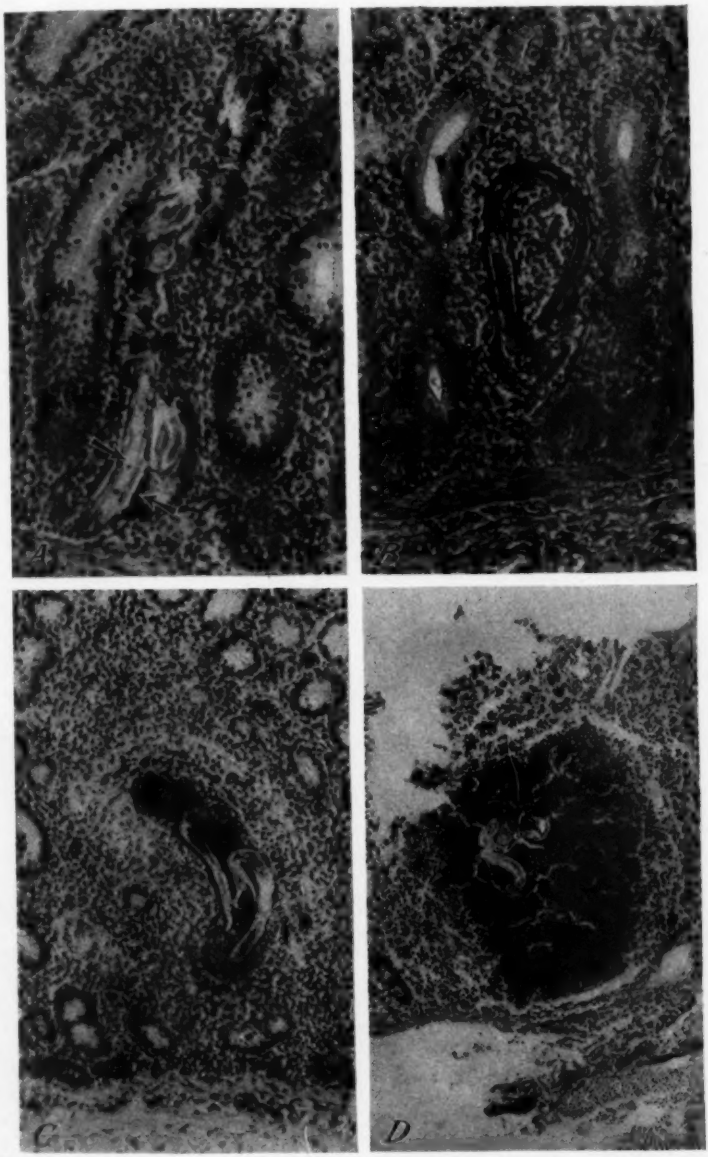

FIGURE 3.—Stages in formation of nodules containing *Cooperia curticei* larvae in the crypt of the intestinal mucosa of a resistant lamb: *A*, Early cell reaction; *B*, later cell reaction, showing increase in number of leucocytes and destruction of crypt wall; *C*, nodule formation showing beginning of necrosis of the central portion of the nodule; *D*, older nodule showing necrotic center containing a larva of *C. curticei*.

immediate vicinity of the larva, produces the condition shown in figure 3, *B*, in which the crypt is destroyed. Later, through the disintegration of the cells in the center, the lesion there becomes an amorphous, necrotic mass, and a recognizable nodule begins to form (fig. 3, *C*). Figure 3, *D*, is an illustration of an older nodule containing a large necrotic center enclosing a nematode larva. That the occurrence of these nodules was apparently related to the development of acquired resistance to superinfection is shown by the data given in table 1.

TABLE 1.—*Data on experimental infections with Cooperia curticei in seven lambs*

Lamb No.	Dates on which larvae were given	Larvae per dose	Doses given	Total larvae given	Days from first administration of larvae to death of host	Worms recovered	Worms recovered in terms of larvae given	Nodules in mucosa of intestine
		Number	*Number*					
88	Nov. 17	10,000	1	*Number* 37,500	*Number* 25	*Number* 7,213	*Percent* 19.23	*Number* 0
	Nov. 22	2,000	1					
	Nov. 27	10,000	1					
	Dec. 1	5,000	1					
	Dec. 5	3,000	1					
	Dec. 7	3,000	1					
	Dec. 9	4,500	1					
102 [1]	Aug. 3–29	5,000	27	135,000	88	25,033	18.54	0
118	Aug. 27	1,000,000	1	1,000,000	5	95,960	9.60	0
63 [1]	June 18–Sept. 9	1,000	84	105,000	126	6,919	6.59	319
	Sept. 10–Oct. 21	500	42					
61 [1]	June 18–Oct. 21	500	126	63,000	126	8,018	12.73	1,943
65 [1]	July 16–Sept. 9	250	56	66,000	125	455	.007	3,273
	Sept. 10–Oct. 15	500	36					
	Oct. 16–Nov. 18	1,000	34					
103 [1]	Aug. 11–Aug. 14	100,000	4	400,000	16	0	.000	32,000

[1] Doses were given daily to this animal for period or periods shown.

The data in this table show that nodules occurred only in lambs that received daily doses of larvae over a relatively long period and in lamb 103, which had been infected during the preceding summer. Furthermore, the presence of the nodules appeared to be associated with a decrease in the percentage of worms recovered post mortem. At first glance the data for lambs 61 and 118 do not appear to bear out this statement. However, in lamb 61 the relatively high percentage of larvae recovered can be explained by the lower dose rate, which apparently resulted in the establishment of a large number of larvae before the resistance of the lamb was built up sufficiently to interfere with their development. In lamb 118, the small percentage of larvae recovered was undoubtedly due to the fact that, at the time of autopsy, most of the larvae were within the crypts of the mucosa, and, being difficult to wash out, they remained in the intestine. That this explanation is probably the correct one is borne out by the finding of numerous larvae in cross sections of the intestine of this lamb. At the time this work was done the importance of the total number of nodules was not appreciated, and the intestine of lamb 63 was placed in 10-percent formalin for preservation overnight before all the nodules were counted. This treatment rendered all but the largest nodules invisible and resulted in the small number reported from this lamb.

The fact that a smaller percentage of larvae were recovered from those lambs having nodules in the intestine indicates a definite rela-

tionship between nodule formation and the resistance of the host to infection with this parasite. The occurrence of this phenomenon in connection with the superinfection of sheep with *C. curticei* is of significance since the larvae of this nematode, so far as could be determined from sections of tissue examined, do not penetrate into the mucosa of the host. Because of this fact they have slight contact with the host's tissue, yet they are able to sensitize the intestinal mucosa. As a result, on subsequent infection the larvae are presumably immobilized in the crypts of the intestinal mucosa and are prevented from returning to the lumen where they would be able to complete their development to maturity.

The finding of worm nodules in the intestinal wall of domestic ruminants is not new. In 1876 Drechsler (*7*) described worm nodules in the wall of the small intestine of a cow that he had examined for lesions of tuberculosis. This finding was confirmed by Saake (*33*) the following year. The first report of worm nodules from the intestine of cattle in the United States was made by Smith in 1894 (*38, pp. 124–127*).

Curtice (*5*) first described nodules in the intestine of sheep in 1890, and in 1892 Giles (*11, pp. 45–49*) confirmed Curtice's finding in a report on worm nodules from sheep in India.

Since these reports, Gips (reported by Roloff and Schütz (*32*)), Janson (*14*), Ströse (*39*), Von Rátz (*31, pp. 230–232*), Scheben (*35*), Marotel (*23*), Ebhardt (*8*), Jowett (*16, 17*), Bontz and Krause (*3*), Monnig (*25*), and Liebsch (*22*) have described nodular lesions from the intestine of cattle caused by nematode larvae, and Janson (*15*), Liebe (*21*), Scheben (*35*), Joest (*15*), Theiler (*41*), Veglia (*44*), and Fourie (*10*) have described similar lesions from the intestine of sheep. Other workers have reported worm nodules from gallinaceous birds, horses, swine, monkeys, anthropoid apes, and man.

Until recently, however, the significance of the formation of nodules around nematode larvae that have invaded the tissues of the host has been obscure. Veglia (*44*) found that some of the larvae of the nodular worm of sheep (*Oesophagostomum columbianum*) failed to escape from the nodules at the end of the 5-day period, which is the usual duration of the tissue-migration phase in the life cycle of this nematode. Although Veglia associated this delay with the age of the host, he stated that purely from an anatomical point of view an encysted larva, situated too deeply in the intestinal wall or too thickly encased in an adventitious fibrous covering, might not emerge at all, or that its exit might be indefinitely delayed. Monnig (*26*) called attention to the fact that the larvae of *O. columbianum* caused a marked tissue reaction only in sheep that appeared to possess some degree of resistance to the parasite. This resistance was evidenced by the fact that the sheep which showed numerous nodules in the intestine usually had very few or no adults in the colon. Fourie (*10*) also concluded that the tissues in sheep resistant to infestation with *O. columbianum* might succeed in imprisoning the parasite.

Kerr (*18*), working with *Ancylostoma caninum* infestations in mice, showed that resistance developing in this host by previous infestation was intimately associated with great accumulations of leucocytes about the larvae, this phenomenon resulting in the retention and walling off of the latter in the tissues of the host. Kerr (*19*) found

similar cellular reactions about the larvae of *Ascaris lumbricoides* in the mucosa of the ceca of guinea pigs made resistant by previous infection. Sarles and Taliaferro (*34*) and Taliaferro and Sarles (*40*) found similar cellular reactions about the larvae of *Nippostrongylus muris* in the tissues of immune rats. These authors believe that the larvae of this nematode are first immobilized by the action of a specific antibody produced in the tissues of the host as a result of previous infection. The immobilization of the larvae can be accomplished by the introduction into the host of immune serum, according to these writers. After immobilization, polymorphonuclear leucocytes accumulate about the larvae as in ordinary inflammation. These cells soon disintegrate, and with the advant of the macrophages, some of which form giant cells, and the lymphocytes, the lesion takes on the appearance of a typical foreign body reaction.

DISCUSSION

The formation of nodules in the intestinal mucosa, as has been described, is significant in the light of the preceding study of the life history. In no instance were the larvae observed to penetrate the mucosa of the intestine. They came in contact only with the epithelium of the crypts, and yet this contact was sufficiently intimate to sensitize the mucosa to the presence of larvae belonging to a later infestation and thus bring about their destruction.

Histologically, these nodules were identical with those described by early workers, and all of them were very similar to those described by Kerr, Taliaferro, and Sarles, who definitely connected the development of resistance and the occurrence of such tissue reactions with parasitic invasion.

SUMMARY

The life history of *Cooperia curticei* as determined experimentally was found to be very similar to the life history of *Trichostrongylus* spp. as reported by Monnig. When cultured at room temperature, the eggs were found to hatch about 20 hours after being passed in the feces, and the infective larvae developed about 90 hours after hatching. The infective larvae lost their sheaths soon after entering the host and migrated into the crypts of the anterior portion of the small intestine about 3 days after entering the host. However, they were never found to penetrate into the mucosa. They underwent the third ecdysis in the crypts of the mucosa on the fourth day after entering the host, grew rapidly, and returned to the lumen of the gut on about the fifth day. They continued to grow rapidly and passed through the fourth ecdysis on the ninth day after infection and matured on about the fourteenth day after infection. The eggs were found in the feces on the fifteenth day after infection.

Sex differentiation in *Cooperia curticei* was observed at the beginning of the fourth stage.

Lambs receiving daily doses of larvae over a relatively long period and one lamb that had been infected with *C. curticei* during the previous summer developed a resistance against superinfection with the nematode. This resistance was evidenced by the production of nodules about the larvae in the intestinal mucosa of the host. These nodules were found to be similar in structure to those reported by other workers.

The development of these nodules in connection with infestations with *C. curticei* was significant in that the larvae were not observed to actually penetrate the tissues of the host, yet they came into sufficiently close contact with the tissues to sensitize them to subsequent infestations.

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THE VITAMIN G CONTENT OF SOME OIL PRESS-CAKE MEALS AND RELATED PRODUCTS[1]

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INTRODUCTION

Originally the assays reported in this paper were qualitative tests of the relative amounts of vitamin G in peanut meal, cottonseed meal, soybean meal, and linseed meal by the method described by Sherwood and Halverson (*15, p. 854*).[2] Later dried brewers' yeast[3] was adopted as a standard and fed in parallel with the meal being tested in order to evaluate the sample in terms of this yeast.

REVIEW OF LITERATURE

Sometime after the inception (1930) of the work reported herein, Bourquin and Sherman (*4*) published a method for the quantitative estimation of vitamin G, which has been widely used and which has been shown to be essentially an assay of riboflavin (*2, 3, 5*). György (*8*), however, found that some factor in addition to riboflavin is necessary for growth of rats on the Bourquin-Sherman basal ration.

The outstanding difference between the Bourquin-Sherman basal ration and the one used by the present writers is that the former contains an extract of wheat as a source of vitamin B_1 whereas in the latter this vitamin was supplied in the form of an extract of rice polish, tikitiki. Morgan, Cook, and Davison (*12*) state that tikitiki is probably chiefly lacking in flavin. Bender, Ansbacher, Flanigan, and Supplee (*1*) have shown that their rice-polish concentrate contains the antidermatitis factor as well as vitamin B_1. It is reasonable, therefore, to assume that the results reported by the present writers are largely assays of riboflavin, although the influence of other members of the vitamin G complex has not been rigidly excluded and it is possible that suboptimal amounts of one or more of these uncontrolled factors may have influenced the results. The assays are reported, not as absolute values of a single vitamin, but more indefinitely as vitamin G, chiefly riboflavin.

The oil press-cake meals assayed were samples of the commercial products. Their vitamin B_1 content has been reported elsewhere (*16*).

There are few published quantitative vitamin G assays of meals from oily seeds or of the seeds themselves. Morrison (*13*) gave the vitamin G in soybeans and soybean meal a rating of 1+. Scheunert and Schieblich (*14*) stated that fresh soybeans, grown near Leipzig, contain about one-eighth as much vitamin B_2 as high-grade dried brewers' yeast. Wilgus, Norris, and Heuser (*18*) found by tests with chicks that the vitamin G in five samples of soybeans was about 3 percent of that in their standard pork liver, or about 15 percent of

[1] Received for publication November 7, 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 794.
[3] Powdered dehydrated yeast, Northwestern Yeast Co.

that in dried skim milk. They also found that there was no significant difference in vitamin G in the raw seeds and the meals made from them by either the hydraulic, the expeller, or the solvent methods. Levine and Remington (*10*) found 2.4 to 3.2 Sherman units of vitamin G per gram of soybeans and 2.9 units per gram of cottonseed meal. In Daniel and Munsell's (*6*) summary of the vitamin assays of foods, soybeans are listed as containing 900 Sherman units of vitamin G per 100 g, and the same amount of cottonseed flour is stated to contain 170 units. Jukes (*9*) reported that peanut meal contains approximately 1.1 modified Sherman units per gram.

EXPERIMENTAL PROCEDURE

Albino rats, 22 to 26 days old, were given the vitamin B-complex-free basal ration previously described by Sherwood and Halverson (*15*, *16*), with distilled water, and 2 drops daily of an extract of rice polish, prepared by the method of Wells (*17*). When they had ceased to gain in weight, the basal ration was supplemented daily with a weighed amount of the product being tested or of the standard yeast, for a period of 10 weeks. The rats were housed separately in cages having raised screen bottoms.

LENGTH OF TEST PERIOD

Since the rate of gain may decrease because of the depletion of body stores of some nutritive essential not supplied in the supplement or basal ration, Bourquin and Sherman (*4*) warn against prolonging the feeding period beyond the time when the growth curves begin to flatten. The records of the present writers showed that when comparatively large amounts of the test material were fed, few of the curves flattened in 10 weeks; but when smaller amounts were fed, there was a tendency for some of the curves to break after about 7 weeks.

The coefficients of variation in gain of replicate rats getting yeast, while rather variable, usually were largest during the early part of the test period. After 4 or 5 weeks the coefficients of variation tended to reach a minimum and to remain more or less constant throughout the rest of the 10 weeks, thus indicating that the feeding period should be at least 4 weeks.

These observations, together with the consideration that in general, the longer the test period the greater the difference between comparison groups, determined the selection of the gain in 7 weeks as the criterion for judging the results.

CURVES OF REFERENCE

In order to evaluate the vitamin G in terms of yeast equivalent, in those meals which were tested before the reference yeast was adopted, a curve of reference was calculated from the results of all rats getting yeast. This curve, a straight line fitted by least squares, was found to be

$$\text{milligrams of yeast per day} = 34.14 + 1.04 \text{ (gain in 7 weeks)}$$

The standard error was 17.2, and the coefficient of correlation was 0.58.

Because of the comparatively large standard error and the low coefficient of correlation, the data were examined to see if the variation could be lessened by the introduction of corrections for other factors. First-approximation curves, drawn by the short-cut method for multiple curvilinear correlation, as described by Ezekiel (*7, ch. 16*), indicated that the time of year in which the assay was started, the initial weight of the rat, and the gain in the depletion period all influenced the gain response on a given dosage of yeast. These curves also indicated that when the above factors are considered the sex of the rat, the age at weaning, the length of the depletion period, and the weight at the end of the depletion period are of negligible importance.

The estimating curves from the completed multiple curvilinear analysis are shown in figure 1.

These curves are based upon the data from 137 rats; the details, however, are omitted for the sake of brevity. The index of multiple correlation, 0.76, was considerably higher, and the standard error, 13.6, appreciably lower than the corresponding statistics from the linear correlation. This standard error is of approximately the same order of magnitude as that found by Lindholm (*11*), although the two are not strictly comparable because he used a semilogarithmic equation for his curve of reference and expressed his error in terms of percent.

RESULTS OF ASSAYS

Two samples of dried brewer's yeast were checked against the standard yeast. One was a second lot of the same brand as the standard and the other was from a different source.[4] No significant difference was found in the vitamin G content of these three lots, which averaged 19.6 Sherman-Bourquin units per gram.

The results of the assays are shown in table 1. Three estimates of the vitamin G per gram of product are given. The first was obtained by direct comparison of the gain of the rats getting the product being assayed with that of their controls receiving yeast. This cannot be given for assays run before the standard yeast was used. The second estimate was derived by means of the curve of reference and the correction curves of figure 1. Both of these estimates are expressed in terms of milligrams of yeast required to give the same growth response as 1 g of the product. The last estimate is approximately equivalent to Sherman-Bourquin units and is based upon the amount of the product required to promote an average gain of 3 g per week. In the calculation of this value the actual gains were adjusted for season, initial weight, and gain in the depletion period by means of corrections read from curves obtained by repeating the multiple curvilinear analysis with gain in 7 weeks as the dependent variable.[5] The amount of meal required to produce a total gain of 21 g was then read from the curve, relating the corrected gain to the daily dosage of the meal.

[4] Fleischmann's dried brewers' yeast.
[5] The standard error was 6.1 g, and the index of correlation was 0.85.

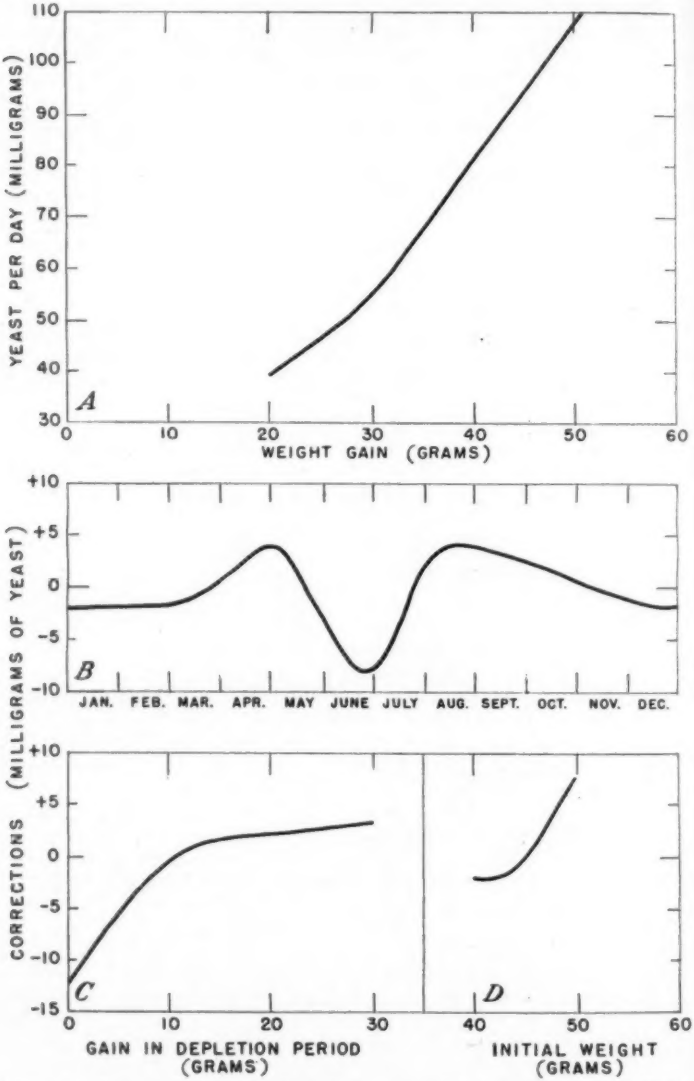

FIGURE 1.—Curves from multiple curvilinear correlation showing: *A*, Relation between the gain in weight of the rats in 7 weeks and the daily dosage of yeast per rat; *B*, correction for season (the month in which the assay was started); *C*, corrections for gain in the depletion period; *D*, corrections for the initial weight of the rats. *B*, *C*, and *D* show how variations in the respective factors affect the gain in weight produced by a given daily dose of yeast.

TABLE 1.—*Vitamin G assays of various feeding materials as estimated by 3 different methods*

Sample No.	Material	Amount fed daily	Rats	Vitamin G per gram		
				As milligrams of yeast (yeast equivalent)—		Sherman-Bourquin equivalent
				By comparison with controls	From curve of reference	
		Grams	*Number*	*Milligrams*	*Milligrams*	*Units*
1	Meal from Spanish peanuts	0.3	3		145	3.7
		.5	3		192	
	Mean				169	
2	Meal from Spanish peanuts	.4	5	102	115	2.5
		.5	6		119	
		.8	5	101	121	
	Mean			102	118	
3	Meal from Virginia Runner peanuts	.3	3		97	2.9
		.5	3		132	
	Mean				115	
4	Meal from Virginia Runner peanuts	.4	6	150	145	2.5
		.5	4	176	154	
		.8	4	138	133	
	Mean			155	144	
5	Cottonseed meal, 36 percent protein	.3	5		112	3.3
		.5	5		127	
		.75	4		120	
	Mean				120	
6	Cottonseed meal, 36 percent protein	.3	6	147	127	3.6
		.5	6	150	126	
	Mean			149	127	
7	Cottonseed meal, 36 percent protein	.3	6	143	137	4.6
		.5	6	134	130	
	Mean			139	134	
8	Cottonseed meal, 36 percent protein	.3	8	117	150	3.3
		.5	8	152	140	
	Mean			135	145	
9	Cottonseed meal, 36 percent protein	.3	8	127	160	4.3
		.5	8	120	116	
	Mean			124	138	
10	Cottonseed meal, 41 percent protein	.3	9	140	163	5.5
		.5	10	184	178	
	Mean			162	171	
11	Cottonseed flour (Allison flour)	.3	3		170	[1] 4.1
12	Cottonseed-hull bran	.3	9	104	150	[2] 2.8
		.5	10	32	82	
	Mean			68	116	
13	Linseed meal	.3	8	117	150	3.3
		.5	7	124	114	
	Mean			121	132	

[1] Calculated from the yeast equivalent.
[2] Calculated from the mean yeast equivalent.

TABLE 1.—*Vitamin G assays of various feeding materials as estimated by 3 different methods*—Continued

Sample No.	Material	Amount fed daily	Rats	Vitamin G per gram		
				As milligrams of yeast (yeast equivalent)—		Sherman-Bourquin equivalent
				By comparison with controls	From curve of reference	
		Grams	*Number*	*Milligrams*	*Milligrams*	*Units*
14	Linseed meal	0.3	8	133	153	3.3
		.5	8	122	114	
	Mean			128	134	
15	Linseed meal	.3	9	33	117	2.5
		.5	9	103	110	
	Mean			68	114	
16	Soybean meal	.5	3	162	[1] 4.1	
17	Soybean meal	.3	8	77	140	3.1
		.5	8	120	148	
	Mean			99	144	
18	Soybean meal	.3	8	117	183	2.8
		.5	8	102	118	
	Mean			110	151	
19	Soybean meal	.3	8	170	160	3.8
		.5	8	140	158	
	Mean			155	159	
20	Soybean meal, solvent process	.3	8	73	190	5.9
		.5	8	164	186	
	Mean			119	188	
21a	Soybeans, Mammoth Yellow, stored whole, 1 year in refrigerator.	.3	8	137	143	3.5
		.5	8	124	114	
	Mean			131	129	
21b	Soybeans, Mammoth Yellow, stored ground, 1 year in refrigerator.	.3	8	137	147	3.7
		.5	8	140	134	
	Mean			139	141	

[1] Calculated from the mean yeast equivalent.

DISCUSSION

In Sample 8 there is poor agreement between the yeast equivalents as determined by direct comparison with the controls, for the two levels of meal fed. When the yeast equivalents are calculated from the curve of reference this discrepancy is largely eliminated, but in sample 9 the difference between the results from the two dosages is enhanced by the comparison with the reference curve. In sample 15 the agreement between the yeast equivalents as determined by the two methods is poor. These are cited as examples of several other similar discrepancies in table 1. If the amount of yeast which contains 1 Sherman-Bourquin unit is calculated by dividing the mean yeast equivalent, from the curve of reference, by the Sherman-Bourquin units for each sample, the values found range from 29 mg (sample 7) to 56 mg (sample 4), whereas figure 1, *A* indicates a value of 41 mg.

So far as the immediate computations are concerned, these discrepancies are caused by differences in the slopes of the curves of response of the rats getting the yeast or the various products. Increasing the dosage level of any of these substances not only increases the riboflavin but also alters both the absolute amounts and the relative proportions of the other components of the vitamin G complex, of vitamin B_1, of protein, and of other factors which may affect the growth rate. Since no two of the products tested are identical in respect to all of these factors, it is not surprising that the slopes of the various curves of response differ.

If the Sherman-Bourquin units had been calculated from the results of that level of product which produced a gain nearest to 3 g per week, the results from the other levels being ignored, the values would have been somewhat different from those in table 1. Use was made, however, of the information obtained from feeding two or more levels by computing these units from the curve of reference as described above.

The errors (variations) of this method of assay are of two kinds: (1) Individual variation of the rats; and (2) variations in the slopes of the curves of response. An estimate of the first, the standard error of the curve of response, has been given. While the second has not been evaluated the discrepancies noted in table 1 give some idea as to its magnitude. When both types of variation are considered, there is no evidence of a significant difference in the vitamin G content of the several products. There is, however, sufficient uniformity to justify the conclusion that where comparisons are possible, the values found are in good general agreement with those given by other investigators.

If the Sherman-Bourquin values in the last column of table 1 are compared with the vitamin G content of foods as shown in Daniel and Munsell's (*6*), table 3, it is seen that peanut meal, cottonseed meal, linseed meal, soybean meal, and soybeans are good sources of vitamin G. They contain less than kidney, liver, or dried milk, which are exceptionally rich in this vitamin, but compare favorably with cheddar cheese, broccoli, beet tops, kale, and turnip greens.

SUMMARY

The term "vitamin G" is used to denote the undifferentiated complex which stimulates the growth of rats on a basal ration containing cornstarch and supplemented with an extract of rice polish but which is otherwise free of water-soluble vitamins. Riboflavin is probably the chief limiting factor in this diet.

Peanut meal, linseed meal, cottonseed meal, soybean meal, and soybeans are good sources of vitamin G, the values ranging from 2.5 to 5.9 Sherman-Bourquin units per gram. They are as rich in this factor as are cheddar cheese and the green leafy vegetables.

The optimum length of the test period was about 7 weeks. The relation between gain in weight of each of the albino rats used in the experiment and their vitamin G intake (as dried brewers' yeast) was affected by the time of year in which the assay was made, and to a less extent by the initial weight of the rat and the gain in the depletion period.

A curve of reference with corrections for season, initial weight, and gain in the depletion period is given. This curve was found useful in

interpreting the results although certain limitations to its general use are recognized.

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